Docket No. 1855/96

#15

U.S. PATENT AND TRADEMARK OFFICE

APPLICATION NUMBER	:	819,305
PATENT NUMBER	:	5,360,897
FILING DATE	:	January 9, 1992
ISSUE DATE	:	November 1, 1994
INVENTION TITLE	:	IMMUNOGENIC CONJUGATES OF STREPTOCOCCUS PNEUMONIAL CAPSULAR POLYMER AND TOXIN OR IN TOXOID[1]
INVENTOR(S)	:	Porter W. Anderson and Ronald J. Eby

Commissioner of Patents and
Trademarks
Washington, DC 20231
Box Pat. Ext.

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. 156

SIR:

The University of Rochester, assignee and owner of the entire 100% interest in U.S. patent 5,360,897 (the '897 patent), through its appointed agent, American Home Products Corporation, submits this request for patent term extension for the '897 patent.

(1) The approved product is Prevnar™ vaccine, a pneumococcal 7-valent conjugate vaccine (Diphtheria CRM $_{197}$ Protein), which is a sterile solution of polysaccharides of the capsular antigens of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F individually conjugated to diphtheria CRM_{197} protein.

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[1]The title of the invention as it appears on the face of the published patent contains a spelling error - it reads "toxiad" instead of the correct term "toxoid."

(2) Regulatory review of Prevnar™ vaccine occurred under section 351 of the Public Health Service Act and/or under 21 U.S.C. § 355, and was designated as a "fast track product" under 21 U.S.C. § 356.

(3) The Prevnar™ vaccine product received permission for commercial marketing under section 351 of the Public Health Service Act and/or under 21 U.S.C. § 355 and § 356 on February 17, 2000.

(4) The active ingredients in the Prevnar™ 7-valent conjugate vaccine are seven individual immunogenic conjugates comprising the polysaccharides of the capsular antigens of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, individually covalently conjugated to the protein carrier CRM_{197}, which is a nontoxic variant of diphtheria toxin isolated from cultures of *Corynebacterium diptheriae* strain C7(β197). Neither the Prevnar™ vaccine, nor its individual immunogenic conjugates have been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act.

(5) This application is submitted by the owner of the '897 patent, the University of Rochester (See copy of assignment, attached as Exhibit A), through its agent, American Home Products Corporation, within the sixty (60) day period permitted for submission pursuant to 37 C.F.R. § 1.720(f). The application was submitted on April 14, 2000, prior to the due date of April 17, 2000.

(6) The patent for which an extension is being sought is U.S. 5,360,897, issued November 1, 1994. The inventors are Porter W. Anderson and Ronald J. Eby. The '897 patent currently expires June 16, 2004.

(7) A copy of the '897 patent is attached hereto as Exhibit B.

(8) A copy of a terminal disclaimer, disclaiming the terminal portion of the '897 patent is attached hereto as Exhibit C. No certificates of correction or reexamination certificates have been issued. A copy of a receipt for maintenance fee payment is provided as Exhibit D.

(9) The '897 patent claims the approved product. The applicable patent claims, and the manner in which each applicable claim reads on the approved product follows:

> Claim 1. An immunogenic conjugate comprising the reductive amination product of an intact capsular polymer of the bacterial pathogen *Streptococcus pneumoniae* having at least two carbonyl groups and a bacterial toxin or toxoid, said conjugate comprising a cross-linked conjugate in which there is a direct covalent linkage between the capsular polymer and the toxin or toxoid.

The Prevnar™ vaccine comprises a sterile solution of seven immunogenic conjugates. Polysaccharides of the capsular antigens of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F individually conjugated, via reductive amination, to a bacterial toxoid comprise the seven immunogenic conjugates. The bacterial toxoid of these seven immunogenic conjugates, CRM_{197}, is a nontoxic variant of diphtheria toxin. *See* Prevnar™ vaccine package insert, at page 1, attached as Exhibit E (a copy of the insert as well as a full text copy is included), and Eby Declaration, at para. 5, attached as Exhibit F.

The polysaccharides of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 19F and 23F are present in the Prevnar™ vaccine product as intact capsular polymers. These polysaccharides are not treated with acid, base, or other reagent which would generate capsular polymer fragments. The polysaccharide of 18C serotype conjugated to CRM_{197} is not an intact polymer. *See* Eby Declaration, at para. 6.

Prior to the reductive amination, the polysaccharides of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F are oxidized with periodate to introduce at least two aldehydes. The aldehyde groups on the polysaccharides are carbonyl groups. *See* Hawley's Chemical Dictionary, eleventh edition, 1993, page 223, attached as Exhibit G. Thus, prior to reductive amination, the polysaccharides contain at least two carbonyl groups. *See* Eby Declaration, at para. 7.

The aldehydes/carbonyl groups on the polysaccharides form, via reductive amination, direct covalent linkages with primary amines (ϵ-amino groups of lysine residues) of CRM_{197}. *See* section 1.6.2.1 of the Drug Master File, attached as Exhibit H. *See* Eby Declaration, at para. 8.

The individual conjugates of the Prevnar™ vaccine product are "cross-linked." The periodate oxidation of the polysaccharide molecules generates multiple aldehydes on each polysaccharide molecule, which react with the multiple amines present on the CRM_{197} molecules. This results in multiple direct covalent linkages. These multiple direct covalent linkages produce cross-linking between the polysaccharides and the CRM_{197} molecules. This cross-linking is evidenced by the fact that the polysaccharide-CRM_{197} conjugate has an increased size/weight over that of the individual polysaccharide and CRM_{197} taken together. *See* Eby Declaration, at para. 9.

> Claim 4. The immunogenic conjugate of claim 1, in which the bacterial pathogen is *Streptococcus pneumoniae* serotype 14.

The package insert states that Prevnar™ vaccine is a solution of the capsular antigens of *Streptococcus pneumoniae* serotype 4, 9V, 14, 18C, 19F and 23F individually conjugated to diphtheria CRM_{197}. Additionally, the package insert indicates that each 0.5 mL dose is formulated to contain: 2µg of each saccharide for serotypes 4, 6B, 9V, 14, 18C, 19F and 23F and 4 µg of serotype 6B.

> Claim 5. The immunogenic conjugate of claim 1, in which the bacterial pathogen is *Streptococcus pneumoniae* serotype 23.

The package insert states that Prevnar™ vaccine is a solution of the capsular antigens of *Streptococcus pneumoniae* serotype 4, 6B, 9V, 14, 18C, 19F and 23F individually conjugated to diphtheria CRM_{197}. Additionally, the insert indicates that each 0.5 mL dose is formulated to contain: 2μg of each saccharide for serotypes 4, 6B, 9V, 14, 18C, 19F and 23F and 4 μg of serotype 6B.

Pneumococcal serotype 23F is the same as serotype 23. The designation "23F" is the nomenclature used in the Danish system for *Pneumococcal* serotype 23. *See* Physicians' Desk Reference, 1993, bottom of page 1262, attached as Exhibit I.

> Claim 6. The immunogenic conjugate of claim 1, in which the toxin or toxoid is diphtheria toxin or toxoid.

The package insert states that the protein carrier CRM_{197} is a nontoxic variant of diphtheria toxin isolated from cultures of *Corynebacterium diphtheriae* strain C7 (β197).

> Claim 7. The immunogenic conjugate of claim 6, in which the toxoid is CRM_{197}.

The package insert states that Prevnar™ vaccine is a solution of the capsular antigens of *Streptococcus pneumoniae* individually conjugated to diphtheria CRM_{197}.

(10) The relevant dates and information pursuant to 35 U.S.C. 156(g) in order to enable the Secretary of Health and Human Services to determine the applicable regulatory review period is:

IND number: 5832

IND effective date: November 25, 1994

NDA number: 99-0279

PLA submission date: March 1, 1999

PLA effective date: June 1, 1999

PLA approval date: February 17, 2000

(11) The Prevnar™ vaccine PLA was approved by the FDA on an IND and PLA filed by Lederle Laboratories ("Lederle"), which is a division of American Cyanamid Company, which is a wholly owned subsidiary of American Home Products Corporation ("AHP"). American Cyanamid Company is the licensee of '897 patent. As a brief description of the significant activities undertaken by Lederle during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities, attached hereto as Exhibit J is a brief chronology of the communications with the FDA during the regulatory review period ending with approval on February 17, 2000. Individual's names and proprietary information has been redacted.

(12) In the opinion of the applicant, the '897 patent is eligible for patent term extension under 35 U.S.C. 156 because

(a) 35 U.S.C. 156(a)
The '897 patent claims a product.

(b) 35 U.S.C. 156(a)(1)
The term of the '897 patent has not expired before submission of this application.

(c) 35 U.S.C. 156(a)(2)
The term of the '897 patent has never been extended.

(d) 35 U.S.C. 156(a)(3)
The application for extension is submitted by the University of Rochester, the owner of record, through its agent, American Home Products Corporation, in accordance with the requirement of 35 U.S.C. 156(d) and rules of the U.S. Patent and Trademark Office.

(e) 35 U.S.C. 156(a)(4)
The Prevnar™ vaccine product has been subject to a regulatory review period before its commercial marketing or use.

(f) 35 U.S.C. 156(a)(5)(A)
The commercial marketing or use of the Prevnar™ vaccine product, after the regulatory review period is the first permitted commercial marketing or use of the Prevnar™ vaccine product, and any of its seven individual conjugates under the provision of section 351 of the Public Health Service Act and/or under 21 U.S.C. § 355 (new drug) and 21 U.S.C. §356 (fast track products) under which such regulatory review period occurred.

(g) 35 U.S.C. 156(c)(4)
No other patent has been extended for the same regulatory review period for the Prevnar™ vaccine product.

The length of extension of the patent term of the '897 patent claimed by applicant is 1086 days, until 6/7/07. The length of the extension was determined as follows.

(a) 1649 The number of days in the period beginning on the date an exemption under section 351 of the Public Health Service Act became effective for the approved product (**November 25, 1994**) and ending on the date the application was initially submitted and effective for such product under section 351 of the Public Health Service Act. (**June 1, 1999**); (See 37 C.F.R. 1.775(c)(1)).

(b)	262	The number of days in the period beginning on the date the application was initially submitted and effective for the approved product under section 351 of the Public Health Service Act, (**June 1, 1999**) and ending on the date such application was approved under such section. (**February 17, 2000**). (See 37 C.F.R. 1.775(c)(2)).
(c)	1911	The sum of (a) and (b). This is the regulatory review period. (37 C.F.R. 1.775(c)).
(d)	0	The number of days in the regulatory review period which were on and before the '897 patent issued. (**November 1, 1994**). (37 C.F.R. 1.775(d)(1)(i)).
(e)	0	The number of days in the regulatory review period during which it is determined under 35 U.S.C. 56 (d)(2)(B) by the Secretary of Health and Human Services that applicant did not act with due diligence.[2] (37 C.F.R. 1.775(d)(1)(ii)).
(f)	0	The sum of (d) and (e).
(g)	1911	(c)-(f). (37 C.F.R. 1.775(d)(1)(ii)).
(h)	1086	½ of (a) + (b). (37 C.F.R. 1.775(d)(1)(iii)).
(i)	06/16/04	The original term of the '897 patent, shortened by any terminal disclaimer.
(j)	06/07/07	The original term of the patent as shortened by any terminal disclaimer plus the number of days in (h). (37 C.F.R. 1.775(d)(2)).
(k)	02/17/14	The date of approval of the application under section 351 of the Public Health Service Act, or subsection (b) of section 505 or section 507 of the Federal Food, Drug, and Cosmetic Act plus 14 years. (37 C.F.R. 1.775 (d)(3)). (**February 17, 2000**)
(l)	06/07/07	The earlier of (j) and (k). (37 C.F.R. 1.775(d)(4)).
(m)	06/16/09	(i) plus 5 years. (37 C.F.R. 1.775 (d)(5)(i)).
(n)	06/07/07	The earlier of (l) and (m). (37 C.F.R. 1.775(d)(5)(ii)).

[2]There has been no such determination. To the best of applicant's agent's knowledge, Lederle was diligent during the regulatory review period.

(13) The applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought.

(14) Please charge the prescribed fee for receiving and acting upon this application for patent term extension pursuant to 37 C.F.R. 1.20(j) to deposit account 11-0600.

(15) Please address inquires and correspondence to:

Estelle J. Tsevdos
Kenyon & Kenyon
One Broadway
New York, NY 10004

(16) A triplicate of these application papers is submitted herewith.

(17) The following declarations of Estelle J. Tsevdos of Kenyon & Kenyon, and Mark S. Coburn of the University of Rochester are submitted herewith in compliance with the requirements of 37 C.F.R. § 1.740(b).

DECLARATION

The undersigned, Attorney for the applicant's (University of Rochester) agent, American Home Products Corporation ("AHP")(*See* attached Exhibit K, Power of Attorney to Estelle J. Tsevdos from AHP) which is submitting this application on behalf of the patent owner, the University of Rochester (*See* attached Exhibit L, Grant of Authority from University of Rochester to American Home Products Corporation), for patent term extension of United States Patent No. 5,360,897 herein above referred to as the '897 patent, in compliance with the requirements of 37 C.F.R. § 1.740(b)(1), hereby declares as follows:

1. She is a patent attorney authorized to practice before the United States Patent and Trademark Office (Reg. No. 31145) and she is authorized to represent AHP in this application for patent term extension of the '897 patent and to transact all business in the United States Patent and Trademark Office in connection therewith;

2. She has reviewed and understands the contents of this application for patent term extension of the '897 patent;

3. She believes that the '897 patent is subject to patent term extension pursuant to provisions of 37 C.F.R. § 1.710;

4. She believes that the extension of the length claimed in this application for patent term extension of the '897 patent is justified under 35 U.S.C. § 156 and the applicable regulations relating thereto; and

5. She believes that the '897 patent, which is the subject of this application for patent term extension, meets the conditions for patent term extension as set forth in 37 C.F. R. § 1.720.

Respectfully submitted,

Dated: April 13, 2000

Estelle J. Tsevdos

Estelle J. Tsevdos
Reg. No. 31145

Attorney for Applicant's Agent
American Home Products Corporation

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DECLARATION

The undersigned, Mark S. Coburn, Acting Director, Office of Technology Transfer of the University of Rochester, acting on behalf of the University of Rochester (See certificate of authority, attached hereto), which is the owner of United States Patent No. 5,360,897 ("the '897 patent") for which this application for patent term extension applies, in compliance with the requirements of 37 C.F.R. § 1.740(b)(1), hereby declares as follows:

1. He authorizes American Home Products Corporation and its attorneys to further this application for patent term extension of the '897 patent and to transact all business in the United States Patent and Trademark Office in connection therewith;

2. He has reviewed and understands the contents of this application for patent term extension of the '897 patent;

3. He believes that the '897 patent is subject to patent term extension pursuant to provisions of 37 C.F.R. § 1.710;

4. He believes that the extension of the length claimed in this application for patent term extension of the '897 patent is justified under 35 U.S.C. § 156 and the applicable regulations relating thereto; and

5. He believes that the '897 patent, which is the subject of this application for patent term extension, meets the conditions for patent term extension as set forth in 37 C.F. R. § 1.720.

The undersigned further states that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of the United States Code and that such willful false statements may jeopardize the term extension for the above referenced patent.

Respectfully submitted,

Dated: 4-13-2000

Mark S. Coburn
Acting Director, Office of Technology Transfer
University of Rochester

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510 Hylan Building
RC Box 270140
Rochester, NY 14627-0140

UNIVERSITY OF
ROCHESTER

Charles E. Phelps
Provost

April 10, 2000

To Whom it May Concern:

Certificate of Authority

Mark S. Coburn, as Acting Director of the Office of Technology Transfer, is authorized to execute all papers in connection with the procurement and issuance of all patents domestic and foreign on behalf of the University of Rochester

Charles E. Phelps, Provost
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3117-031

A S S I G N M E N T

WHEREAS, PRAXIS BIOLOGICS, INC., (hereinafter called "ASSIGNOR"), a corporation organized and existing under the laws of the State of New York and having its principal place of business at 30 Corporate Woods, Suite 300, Rochester, New York 14623, is the owner of the entire right, title and interest in, to and under the invention in IMMUNOGENIC CONJUGATES for which United States Patent Application Serial No. 859,975 was filed on May 5, 1986,

WHEREAS, THE UNIVERSITY OF ROCHESTER, (hereinafter called "ASSIGNEE") an educational institution charted by the State of New York and located at Rochester, New York 14627, is desirous of obtaining the entire right, title and interest in, to and under the said invention and the said application:

NOW, THEREFORE, in consideration of the sum of One Dollar ($1.00) and other good and valuable consideration, the receipt of which is hereby acknowledged, ASSIGNOR, has sold, assigned, transferred and set over, and by these presents does hereby sell, assign, transfer and set over, unto the said ASSIGNEE, its successors, legal representatives and assigns, the entire right, title and interest in, to and under the said invention, and the said United States Application and all divisions, renewals and continuations thereof, and all Patents of the United States which may be granted thereon and all reissues and extensions thereof; and all applications for industrial property protection, including, without limitation, all applications for patents, utility models, and designs which may hereafter be filed for said invention in any country or countries foreign to the United States, together with the right to file such applications and the right to claim for the same the priority rights derived from said United States application under the Patent Laws of the United States, the International Convention for the Protection of Industrial Property, or any other international agreement or the domestic laws of the country in which any such application is filed, as may be applicable; and all forms of industrial property protection, including, without limitation, patents, utility models, inventors' certificates and designs which may be granted for said invention in any country or countries foreign to the United States and all extensions, renewals and reissues thereof;

ASSIGNOR authorizes and requests the Commissioner of Patents and Trademarks of the United States, and any Official of any country or countries foreign to the United States whose duty it is to issue patents or other evidence or forms of industrial property protection on applications as aforesaid, to issue the same to the said ASSIGNEE, its successors, legal representatives and assigns, in accordance with the terms of this instrument;

AND ASSIGNOR HEREBY covenants and agrees that it has full right to convey the entire interest herein assigned, and that it has not executed, and will not execute, any agreement in conflict herewith;

AND ASSIGNOR HEREBY further convenants and agrees that it will communicate to the said ASSIGNEE, its successors, legal representatives and assigns, any facts known to us respecting said invention, and testify in any legal proceeding, sign all lawful papers, execute all divisional, continuing, reissue and foreign applications, make all rightful oaths, and generally do everything possible to aid the said ASSIGNEE, its successors, legal representatives and assigns, to obtain and enforce proper protection for said invention in all countries.

IN TESTIMONY WHEREOF, ASSIGNOR has caused this Assignment to be executed by its duly authorized officer this 12th day of April, 1989.

PRAXIS BIOLOGICS, INC.

ATTEST: _____ By: _____ L.S.

Title _____

State of New York

SS.:

County of _____

On this _____ day of _____, 1989 before me, a Notary Public in and for the State and County aforesaid, personally appeared _____, to me known and known to me to be the person of that name, who being duly sworn did depose and say that he is _____ of Praxis Biologics, Inc. and that he signed the foregoing instrument by authority of the Board of Directors of said corporation.

RECORDED
PATENT & TRADEMARK OFFICE
APR 17 1989

COMMISSIONER OF PATENTS

Notary Public

KAREN M. CAPOZZA
Notary Public, State of New York
Qualified in Monroe County
Commission Expires April 14, 19__

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US005360897A

United States Patent [19]

Anderson et al.

[11] Patent Number: 5,360,897

[45] Date of Patent: * Nov. 1, 1994

[54] IMMUNOGENIC CONJUGATES OF STREPTOCOCCUS PNEUMONIAL CAPSULAR POLYMER AND TOXIN OR IN TOXIAD

[75] Inventors: Porter W. Anderson; Ronald J. Eby, both of Rochester, N.Y.

[73] Assignee: The University of Rochester, Rochester, N.Y.

[*] Notice: The portion of the term of this patent subsequent to Jun. 16, 2004 has been disclaimed.

[21] Appl. No.: 819,305

[22] Filed: Jan. 9, 1992

Related U.S. Application Data

[63] Continuation of Ser. No. 423,081, Oct. 18, 1989, Pat. No. 5,097,020, which is a continuation of Ser. No. 859,975, May 5, 1986, Pat. No. 4,902,506, which is a continuation-in-part of Ser. No. 511,048, Jul. 5, 1983, Pat. No. 4,673,574, which is a continuation-in-part of Ser. No. 298,102, Aug. 31, 1981, abandoned.

[51] Int. Cl.[5] A61K 39/385; C07K 17/02

[52] U.S. Cl. 530/403; 530/405; 530/406; 530/807; 424/197.11; 424/244.1; 424/831; 424/832

[58] Field of Search 530/395, 405, 406, 807, 530/403; 424/92, 88

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(List continued on next page.)

Primary Examiner—Kay K. Kim
Attorney, Agent, or Firm—Pennie & Edmonds

[57] ABSTRACT

An immunogenic conjugate which is the reductive amination product of an immunogenic capsular polymer fragment having at least one reducing group and derived from a bacterial capsular polymer of a bacterial pathogen, and a bacterial toxin or toxoid. The invention also relates to methods for the preparation of the conjugates, a vaccine containing the conjugates which elicits effective levels of anti-capsular polymer antibodies in humans. Also disclosed are methods for inducing active immunization against systemic infection in young mammals caused by bacterial pathogens comprising the administration of an immunogenic amount of the above-described conjugate. In a preferred embodiment, the capsular polymer fragment prior to conjugation has at least one aldehyde group at each end of the fragment. The final conjugate made with such capsular polymers has a lattice or network structure, and provides extremely high levels of anti-capsular polymer antibodies in infants.

7 Claims, No Drawings

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IMMUNOGENIC CONJUGATES OF STREPTOCOCCUS PNEUMONIAL CAPSULAR POLYMER AND TOXIN OR IN TOXIAD

This is a continuation of application Ser. No. 07/423,081, filed Oct. 18, 1989, now U.S. Pat. No. 5,097,020, which is a continuation of application Ser. No. 06/859,975 filed May 5, 1986, currently U.S. Pat. No. 4,902,506, which is a continuation-in-part of application Ser. No. 06/511,048 filed Jul. 5, 1983, currently U.S. Pat. No. 4,673,574, which is a continuation-in-part of application Ser. No. 06/298,102, filed Aug. 31, 1981, currently abandoned, all of which are incorporated by reference herein.

FIELD OF THE INVENTION

This invention relates to the field of novel vaccine compositions, processes for producing them and methods for immunization of young warm-blooded animals, including humans, against infections and disease caused by bacteria, including, for example, *Haemophilus influenzae* type b, *Escherichia coli, Neisseria meningitidis* serogroups A and C, *Streptococcus pneumoniae* serotypes 3, 6, 12, 14, 19, 23 and 51, and *Pseudomonas*.

BACKGROUND OF THE INVENTION

It is known that purified bacterial capsular polymers (CP) generally are immunogenic in mature humans and animals and can be used as vaccines against the corresponding systemic infections. As used in this application, the term "capsular polymers" refers to sugar-containing polymers, such as polymers of sugars, sugar acids, amino sugars, polyhydric alcohols and sugar phosphates, and does not refer to amino acid-containing polymers. These "capsular polymers" are frequently referred to in the medical literature as "capsular polysaccharides", though they may contain linkages other than glycosidic linkages and constituents other than sugars such as those listed above.

The capsular polymers of different bacteria vary widely in immunogenicity in the first year of human life. Some are moderately active, such as *Streptococcus pneumoniae* serotype 3 and *Neisseria meningitidis* serogroup A. The susceptibility to systemic infection by encapsulated bacteria is greater in the first year of life. The immunogenic response to many bacterial capsular polymers in children is age dependent, i.e., immunocompetence to CP increases to adult levels by about six years of age.

Among the inactive CP are those of *Haemophilus influenzae* type b, *Streptococcus pneumoniae* serotypes 6 and 12, and *Neisseria meningitidis* serogroup C. Examples of CPs which give an intermediate response in infants are *Streptococcus pneumoniae* serotypes 19 and 51.

INTACT CAPSULAR POLYMERS AS ANTIGENS IN VACCINES

Various investigators have isolated and purified intact capsular polymers which may be useful in or as vaccines. For example, U.S. Pat. No. 4,220,717 describes a process for the isolation and purification of immunologically active polyribosyl ribitol phosphate (PRP) from the capsular polymer of *H. influenzae* b. Additionally, U.S. Pat. No. 4,210,641 relates to polysaccharide extracts of *H. influenzae* having an apparent molecular weight greater than 200,000 daltons and composed principally of galactose, glucose and mannose and containing a small amount of osamines.

Several researchers have utilized these and other intact capsular polymers in formulations to achieve better immunological responses. For example, U.S. Pat. No. 4,196,192 discloses a vaccine containing purified intact PRP and whole *Borderella pertussis* bacteria. This approach to increasing immunogenicity resulted in enhanced levels of anti-PRP and anti-pertussis antibodies in young mammals.

VACCINES CONTAINING CONJUGATES

Other researchers have studied conjugation of capsular polymers to carrier proteins in an effort to enhance antibody formation by the so-called "carrier effect". For example, Schneerson et al., J. Exper. Med. 152:361-376 (1980), describes *H. influenzae* b polymer-protein conjugates disclosed to confer immunity to invasive diseases caused by *H. influenzae* b. The reference documents the age-related immunological behavior of capsular polymers in infants and seeks to overcome this age-dependence by conjugation of the intact capsular polymer with a variety of proteins, including serum albumins, *Limulus polyphemus* hemocyanin and diphtheria toxin. The method of conjugation involves the use of a linking agent such as adipic dihydrazide.

Geyer et al., Med. Microbiol. Immunol. 165:171-288 (1979), prepared conjugates of certain *Klebsiella pneumoniae* capsular polysaccharide fragments to a nitrophenylethylamine linker by reductive amination, and the derivatized sugar was then attached to proteins using azo coupling.

SUMMARY OF THE INVENTION

The present invention relates to the covalent attachment of capsular polymer fragments derived from bacterial capsular polymers to bacterial toxins or toxoids by means of reductive amination. As used in the present application, the term "toxoid" means a form of a toxin which has the antigenicity of the toxin without its toxicity.

The immunogenic conjugates of the invention are prepared by first forming reducing end groups on fragments of the capsular polymers and reacting these with amine groups of the bacterial toxin or toxoid by reductive amination. The reducing end groups may be formed by any suitable method, including selective hydrolysis, e.g., by acids or enzymes, or by oxidative cleavage, e.g., by periodate or related oxygen acids. The conjugation is preferably achieved by reductive amination in an aqueous solution containing cyanoborohydride anions.

The immunogenic conjugates of the invention may be formulated with a pharmaceutically acceptable carrier to produce a vaccine which elicits effective levels of anticapsular antibody formation in young mammals, including humans. The vaccine may be utilized to induce active immunization against systemic infection in young mammals caused by the respective encapsulated bacteria by administering an immunogenic amount of the conjugate to the mammal.

The immunogenic conjugates have been found to be less age dependent than the capsular polymers alone, and are useful for the active immunization of very young warm-blooded mammals against systemic infections by the respective encapsulated bacteria.

Furthermore, the immunogenic conjugates of the invention do not contain potentially toxic linking

agents, such as adipic dihydrazide or p-nitro-phenylethylamine, which have been used in conjugating carbohydrate to protein.

Finally, the immunogenic conjugates of the invention contain fragments of capsular polymers, not intact capsular polymers. The highly repetitive structure of capsular polymers may be in part responsible for their failure to expand the capacity for antibody production in infants. A conjugate of intact (highly polymerized) CP and protein may only partially overcome the immunologic disadvantages of CP alone.

On the other hand, the use of capsular polymer fragments on a carrier may circumvent the disadvantages of the repetitive structure. Additionally, the CP determinants of a conjugate having CP fragments are on the average closer to the carrier than are the CP determinants of conjugates having intact CP, and this proximity to carrier may be necessary for a more effective "carrier effect".

A further advantage lies in the use, for the protein carrier, of a bacterial toxin or toxoid against which children are routinely vaccinated, e.g., tetanus or diphtheria. Desired immunity to the toxin or toxoid is induced along with immunity against the pathogens associated with the capsular polymer.

It is to be understood that reference throughout this specification to any theory to explain the results described is not to limit the scope of the invention. Independent of the method by which the invention functions, the results and advantages described herein may be achieved by reference to the following detailed description.

DETAILED DESCRIPTION OF THE INVENTION

The conjugates of the invention are formed by reacting reducing end groups of the capsular polymer fragment to primary amino groups of a bacterial toxin or toxoid to yield antigenic determinants of the capsular polymer covalently linked to the carrier protein. The reducing groups may be formed by selective hydrolysis or specific oxidative cleavage, or combinations of both.

Antigenic fragments with at least one reducing end can be generated from capsular polymers by a variety of methods, depending upon the structural features of the particular capsular polymer. Limited oxidative cleavage by periodate (or related reagents such as paraperiodic acid, sodium metaperiodate and potassium metaperiodate) will leave aldehydic termini; such an approach will be limited to polymers having vicinal dihydroxy groups. Hydrolysis of a glycosidic linkage produces a reducing sugar terminus. Such hydrolysis can be most specifically accomplished enzymatically by glycosidases, but this application would be restricted to a relatively few capsular polymers, e.g., *Streptococcus pneumoniae* 8, for which glycosidases are known. Acidic hydrolysis is commonly used for hydrolysis of glycosidic linkages. The utility of this approach would be limited if the polymer contains acid-sensitive non-glycosidic linkages or if the polymer contains acid-Sensitive branch linkages important to the antigenic specificity. Base hydrolysis can also be used if the polysaccharide contains base labile bonds to the glycosidic carbon such as phosphate, sulfate or ester linkages. The utility of this approach would be limited if the polymer contained other base sensitive non-glycosidic linkages.

Certain capsular polymers may lack vicinal dihydroxy groups which are susceptible to cleavage by periodate (or related oxygen acids). However, prior hydrolysis of such capsular polymers with acid, base or enzyme may liberate vicinal dihydroxy groups which would be susceptible to oxidative cleavage generally. For example, removal of pyruvic acid, acetates, formates, etc., by acid hydrolysis, or removal of sialic acid by enzyme cleavage, may be done prior to the oxidative cleavage step. This, of course, would be limited in application to those capsular polymers in which the groups modified are not important to antigenic specificity.

Where the capsular polymer is hydrolyzed to form capsular polymer fragments having only one functional aldehyde group, conjugation to a multifunctional protein (having at least two free amine groups) results in a conjugate in which a single molecule of the protein has one or more capsular polymer fragments covalently attached. It can readily be seen that the number of capsular polymer fragments attached to the protein can be routinely regulated by changes in the conditions of the conjugation reaction, including the relative concentration of capsular polymer fragments to protein and the overall concentration of the reactants. Of course, regulation of any reaction parameter, e.g., time, temperature, pH, etc., which affects the reactivity or rate of reaction will alter the final composition and structure of the conjugate.

When the capsular polymer fragment has at least one functional aldehyde group located on each end of the fragment (for example, as the result of oxidative cleavage of vicinal dihydroxy groups of a non-cyclic residue), conjugation to a multifunctional protein can result in several types of conjugate. For example, conjugation of such reactants has the potential for forming a lattice or network structure, particularly where there are many free amines on the protein and capsular fragments are in low molar excess to protein. The degree of cross-linking and overall size of the network or lattice can be regulated. by routine variation of the conditions of the conjugation reaction.

The conjugation os carried out according to the reductive amination process of Schwartz and Gray, Arch. Blochem. Biophys. 181:542-549 (1977). Briefly, the process involves reacting the reducing capsular polymer fragment and bacterial toxin or toxoid in the presence of cyanoborohydride ions, or another reducing agent which will not reduce the reducing ends of interest nor adversely affect the toxin or toxoid or capsular polymer.

The cyanoborohydride ions (or their equivalent) act primarily as a mild selective reducing agent of the Schiff base intermediate formed between the carbonyl groups of the capsular polymer fragment and amino groups of the protein. A secondary effect of such ions is the slower reduction of any active aldehyde groups remaining on the capsular polymer fragments after conjugation has occurred. Optionally, after conjugation, additional cyanoborohydride ions (or their equivalent) may be added to reduce such unreacted free aldehyde groups. It is often desirable to add the stronger reducing agent, borohydride ion, after conjugation to ensure adequate reduction of the remaining carbonyl groups.

Thus, unlike previously employed conjugation procedures wherein the active molecules are joined by a linking agent which forms a part of the final product, the reducing anions utilized herein are not incorporated into the final product. This is important from the standpoint of controlling the potential toxicity (i.e., undesired immunogenicity) of the final product. Evidence of co-

valent linkage is demonstrated by the fact that the association between, for example, a PRP moiety and the carrier protein persists despite salting-out of the protein in the presence of 8M urea, which has a great ability to disrupt non-covalent bonds.

Suitable carrier proteins are those which are safe for administration to young mammals and immunologically effective as carriers. Safety would include absence of primary toxicity and minimal risk of allergic complications. Diphtheria and tetanus toxoids fulfill these criteria; that is, suitably prepared, they are non-toxic and the incidence of allergic reactions is well documented. Though the risk of allergic reaction may be relatively significant for adults, it is minimal for infants.

In the "carrier effect" a weak antigen, by being attached to a stronger antigen as carrier (e.g., a heterologous protein), becomes more immunogenic than if it were presented alone. If an animal is previously immunized with the carrier alone, it may become "primed" for an enhanced response not only to the carrier antigen but also the attached weaker antigen. Infants are routinely immunized with tetanus and diphtheria toxoids. Thus, they would be primed for subsequent presentation of a capsular polymer antigan conjugated to either of these toxoids.

In general, any heterologous protein could serve as a carrier antigan. However, certain bacterial toxins such as tetanus and diphtheria may have an additional advantage in that they are composed of two portions, one of which (the "binding" subunit) has a strong affinity for binding to mammalian cell surfaces. Conceivably, conjugation to such a "binding" protein would permit the carried antigan to more effectively initiate responses in cells of the immune system.

The carrier proteins to which the capsular polymer is conjugated may be native toxin or detoxified toxin (toxoid). Also, by relatively recent mutational techniques, one may produce genetically altered proteins which are antigenically similar to the toxin yet non-toxic. These are called "cross reacting materials", or CRMs. CRM_{197} is noteworthy since it has a single amino acid change from the native diphtheria toxin and is immunologically indistinguishable from it.

A culture of *Corynebacterium diphtheria* strain C7 (β197), which produces CRM_{197} protein, has been deposited with the American Type Culture Collection, Rockville, Md. and has been assigned accession number ATCC 53281.

Conjugation of capsular polymer to native toxin may reduce toxicity, but significant toxicity may remain. Thus, further detoxification would be required. Conventional detoxification of protein toxins employs formalin, which reacts with free amino groups of the protein. Residual toxicity may still be a concern. Furthermore, spontaneous retoxification is possible with any particular lot of vaccine and remains an issue of concern with this approach.

Alternatively, native toxin may be detoxified with formalin to produce conventional toxoid before conjugation to capsular polymer. However, the prior formalin treatment reduces the number of free amino groups available for reaction with the reducing groups of the capsular polymer fragment. CRMs, thus, have significant advantages in that they have no inherent toxicity yet none of their amino groups is occupied by the formalin. A further advantage is that no biohazards exist in working with CRMs.

In the case of CRM_{197}, which is immunologically identical to native toxin, treatment with formalin (though there is no need to detoxify) greatly enhances the immunological response. It is thought that this is due to stabilization of the molecule against degradation by mechanisms of the body and/or aggregation by cross-linking (immunogenicity of particles increases with size).

For all of the above reasons, tetanus and diphtheria toxins are prime candidates for carrier proteins, yet there are others which may also be suitable. Though these others may not have the history of safety found with diphtheria and tetanus, there may be other overwhelming reasons to use them. For instance, they may be even more effective as carriers, or production economics may be significant. Other candidates for carriers include toxins or toxoids of pseudomonas, staphylococcus, streptococcus, pertussis and enterotoxigenic bacteria, including *Escherichia coli.*

Suitable carrier media for formulating a vaccine include sodium phosphate-buffered saline (pH 7.4) or 0.125M aluminum phosphate gel suspended in sodium phosphate-buffered saline at pH 6 and other conventional media. Other pharmaceutical carriers suitable for use in vaccines are known in the art.

Generally, vaccines of the invention containing from about 5 to about 100 μg, preferably about 10 to 50 μg, are suitable to elicit effective levels of antibody against the capsular polymer in young warm-blooded mammals. Of course, the exact dosage would be determined by routine dose/response experimentation. Several small doses given sequentially would be expected to be superior to the same amount of conjugate given as a single injection.

The vaccines of the invention may be administered by injection to warm-blooded mammals of any age and are especially adapted to induce active immunization against systemic infections in young mammals caused by the pathogens *Haemophilus influenzae* type b, *Escherichia coli,* pneumococcus, meningococcus, streptococcus and pseudomonas.

The following are non-limiting examples of methods for the preparation of exemplary immunogenic conjugates of the present invention and their use in vaccines.

EXAMPLE: GENERATION OF LARGE, MEDIUM AND SMALL FRAGMENTS OF PRP CONTAINING REDUCING END GROUPS AND CONJUGATION TO CRM_{197}

The capsular polymer of *Haemophilus influenzae* type b is a linear polymer with the repeating unit [-3-β-D-ribosyl(1-1) ribitol(5-phosphate)-] (PRP). Generally, acid hydrolysis of PRP is carried out until the ratio of total to reducing ribose has dropped to 25 or below. The resulting mixture of size fragments may be fractionated by molecular sieve column chromatography to isolate the desired size range of fragments for conjugation. The method for obtaining fragments is as follows:

a. A sample of sodium PRP, (nucleic acid content 0.006%) containing 28.6 milligrams ribose was dissolved with distilled water to make a total volume of 9.2 ml in a 125-ml erlenmeyer flask and chilled in ice.
b. 1.02 ml of 0.1N H_2SO_4 was added.
c. Duplicate samples of 0.01 ml of the acidified PRP were transferred to test tubes held on ice (0-minute)

d. The flask was transferred to a boiling-water bath for 3 minutes, then chilled in an ice-water bath.

e. Step c was repeated (3-minute sample).

f. The samples were assayed for reducing power by the alkaline ferricyanide method standarized with D-ribose.

g. Based on the result (see Table 1), step d was repeated.

h. Step c was repeated (6-minute samples).

i. Step f was repeated.

TABLE 1

Samples	Nanomoles of reducing ribose (av)	Ratio, total ribose/ reducing ribose
0-min	0.42	493
3-min	6.08	34.0
6-min	9.66	21.4

The result (see Table 1) indicated that, assuming the sole mode of hydrolysis had been at the (1-1) glycosidic linkage, after 6 minutes the number-average chain length was 21.4 monomeric units, i.e., (ribitol-5-phosphate-3-ribose).

j. 0.102 ml 1N NaOH was added, and the pH was estimated by indicator paper (about pH 6).

k. The neutralized hydrolysate was lyophilized.

l. Bio-Gel P10 (Bio-Rad, Inc.) was equilibrated in 0.1M triethylammonium acetate and poured into a 1.5 cm diameter chromatographic column, giving a gel-bed height of 98 cm.

m. The lyophilized material (step k) was rehydrated with 2.7 ml water, and 0.3 ml of 1M triethylammonium acetate was added. This solution was applied to the column and elution was carried out with collection of 3.5 ml fractions.

n. The elution of ribosyl residues was determined by assay of 0.005-ml samples of each fraction for riboes content by the orcinol reaction with D-ribos. as standard.

o. Fractions were combined into 3 pools, L, M, and S as indicated in Table 2, and the pools were assayed for total ribose and reducing ribose:

TABLE 2

Pool	Fractions contained	Total ribose, micromoles	Ratio, total ribose/ reducing ribose	Est. Mn*	Range of Ve/Vo of fraction
L	15–18	577	31.2	11,000	≦1.08
M	19–23	744	18.6	6800	1.09–1.38
S	24–34	1180	9.1	3400	1.39–1.99

*on the assumption that the sole hydrolysis was glycosidic.

p. The pools were lyophilized, re-hydrated with 10 ml water, re-lyophilized, and re-hydrated with 1.5 ml water. 1.2 ml of the last solutions were transferred to microcentrifuge tubes and lyophilized in preparation for the conjugation reactions.

Conjugation of CRM_{197} to Reducing Fragments of PRP

a. To the microcentrifuge tubes containing lyophilized fragments, L, M, and S, and to an empty tube (C or control) were added potassium phosphate buffer pH 8, 2.7 milligrams CRM_{197}, and 4 milligrams sodium cyanoborohydride, such that the final volume was 0.2 ml and the phosphate-buffer was at 0.2M.

b. The tubes were incubated at 37° C. with daily mixing.

c. After 18 days the tubes were centrifuged 2 minutes at 7000 G.

d. After determination that the majority of protein was in the precipitates, the precipitates were washed four times with ≦1 ml water.

e. The washed precipitates were made 8M in urea and warmed to 50° C., dialyzed against saline overnight at 4° C., and centrifuged. The supernates were separated and made 95% saturated in ammonium sulfate, held overnight at 4° C., and centrifuged. The resulting precipitates were washed 3 times with 0.4 ml of 95% saturated ammonium sulfate, and suspended with 1 ml water. These colloidal suspensions were labeled CRM_{197}-PRP-L, -M, -S, and CRM_{197}-C, respectively.

f. The preparations were assayed for protein by means of the Folin phenol reaction with bovine albumin as standard and for ribosyl residues with the orcinol reaction and D-ribose as standard. The results are given in Table 4. The preparations were assayed for PRP antigenic activity by their ability (at concentrations of 50 micrograms protein/ml) to inhibit the binding of labeled native PRP to human anti-PRP antibody (Table 3).

TABLE 3

Preparation tested	% Antigen bound	antigenic activity, ng PRP equiv./μg protein
none	28.1	—
>native PRP, 0.5 ng	6.7	—
>native PRP, 5 ng	0.94	—
CRM_{197}-C	34.3	0.0
CRM_{197}-PRP-S	2.0	0.1
CRM_{197}-PRP-M	2.5	0.08
CRM_{197}-PRP-L	3.9	0.006

Thus, all the tested conjugates of CRM_{197} with PRP fragments were antigenically active, while the control preparation in which CRM_{197} was exposed to cyanoborohydride in the absence of PRP fragments was inactive as expected.

The preparations were assayed for immunogenicity in rabbits in comparison with high molecular weight purified PRP, and the results are given in Table 4. Rabbits given the PRP control or the CRM_{197}-C control made barely detectable increases in anti-PRP antibody. Rabbits given any of the three CRM_{197}-PRP conjugates made progressive increases after each injection; the titers after the third injection were 1000-fold greater than prior to immunization. In an experiment not illustrated a simple mixture of CRM_{197} and PRP fragment preparation L was assayed in rabbits and found not to elicit anti-PRP antibody.

TABLE 4

ANTI-PRP ANTIBODY RESPONSE TO CONJUGATED AND CONTROL VACCINES OF WEANLING RABBITS PRIMED WITH ORDINARY DIPTHERIA TOXOID*

	Rabbit Vaccine**	Pentose/ protein ratio	Anti-PRP Antibody, ng/ml. at age in weeks			
			7***	8***	9***	10
1	PRP(MW 10^5)		<10	12	28	40
2	"		<10	<10	27	26
3	CRM_{197}-C (control)	—	35	25	31	36
4	"		16	34	40	48
5	CRM_{197}-PRP-S	0.015	19	980	26,000	49,000
6	"		<10	84	23,000	31,000
7	CRM_{197}-PRP-M	0.0069	<10	37	~2,500	11,000
8	"		23	11,000	49,000	150,000
9	CRM_{197}-PRP-L	0.0020	14	73	3,700	26,000
10	"		<10	340	9,800	76,000

*The rabbits were New Zealand Whites obtained from Dutchland Farms immediately after weaning. At six weeks of age each was injected subcutaneously (s.c.) with 40 Lf of diptheria toxoid (Massachusetts Dept. of Public Health) contained in 0.5 ml of 0.0125 M aluminum phosphate pH 6 (alum).
**The PRP vaccine was 30 μg PRP lot 17 contained in 0.1 ml saline. The other vaccines were 25 μg protein contained in 0.5 ml alum.
***Injections of the indicated vaccine were given (s.c.) immediately after bleeding. There were two rabbits per vaccine. Listed are individual titers, determined by radioantigen binding with ^{3}H-labeled native PRP.

The protective potential of the anti-PRP antibodies induced by the conjugates was evaluated by testing the bactericidal activity of the rabbit sera of Table 4. The bactericidal titers were determined against *H. influenzae* b strain Eag by the methods of Anderson et al., J. Clin. Inv., 65: 885–891 (1980). Table 5 shows that before vaccination the sera were unable to kill the bacterial (reciprocal titers <2). After three injections the reciprocal titers of the rabbits receiving the CRM_{197}-PRP conjugates had risen to 16 or greater while titers of the rabbits receiving the CRM_{197} control remain at <2.

TABLE 5

Bacterial Titers Against *H. influenzae* b Strain Eag of Sera of Weanling Rabbits Vaccinated With CRM_{197} or Its Conjugates With Oligosaccharides S, M, and L of PRP*

Rabbit	Vaccine given	Reciprocal serum dilution for >90% Killing: Pre-vaccination	After 3 injections
3	CRM_{197} control	<2	<2
4	CRM_{197} control	<2	<2
5	CRM_{197}-PRP-S	<2	128
6	CRM_{197}-PRP-S	<2	≧256
7	CRM_{197}-PRP-M	<2	16
8	CRM_{197}-PRP-M	<2	64
9	CRM_{197}-PRP-L	<2	64
10	CRM_{197}-PRP-L	<2	32

*Same vaccinations as described in Table 4.

EXAMPLE: VARIATION OF PRP FRAGMENT RATIO TO CRM_{197}

In this example, the ratio of PRP fragment S to CRM_{197} was varied and the conservation of antigenic activity of the CRM_{197} component was examined in addition to the PEP component.

Preparation of CRM_{197}-PRP-S#2, A and B

a. To microcentrifuge tubes A and B were added 0.15 ml each of the solution of fragments S described above, i.e., steps o and p. The solutions were lyophilized.
b. Tube A received 0.015 ml 2M potassium phosphate buffer pH 8, 0.1 ml of CRM_{197} 5 mg/ml in 0.01M sodium phosphate buffer pH 7, and 0.015 ml of sodium cyanoborohydride 200 mg/ml.
c. Tube B received 0.002 ml of the pH 8 buffer and 0.1 ml of the CRM_{197} solution. The resulting solution was lyophilized. The solids were suspended with 0.015 ml water, and 0.002 ml of the pH 8 buffer were added.
d. Tubes A and B were incubated at 37° C. for 13 days. To tube B an additional 0.002 ml of cyanoborohydride was added. Both tubes were incubated at 37° C. for an additional 3 days. (Note that due to the reduced reaction volume, the concentrations of reactants in B were higher than A.)
e. To A was added 0.06 ml water and 0.8 ml saturated ammonium sulfate (SAS). To B was added 0.175 ml water and 0.8 ml SAS.
f. The tubes were incubated 1 hour at 0° C. and centrifuged 20 minutes at 8000 G. The supernates were removed.
g. The precipitates were washed by suspension in 1 ml of 80% SAS, centrifugation at 8000 G 20 minutes, and removal of the supernates.
h. The precipitates were suspended with 0.1 ml water, and 0.4 ml SAS was added.
i. Same as step f.
j. Same as step g.
k. The precipitate in B was dissolved with 0.084 ml 9.5M urea (estimated final concentration 8M); 0.1 ml water and 0.8 ml SAS were added, and the precipitate was isolated as in step f. This precipitate was washed as in step g.
l. The precipitates in A and B were suspended with 0.2 ml water. The suspensions were separated into soluble (s) and insoluble (i) fractions by centrifugation 30 minutes at 8000 G, and the s fractions (supernates) were made 0.01M sodium phosphate buffer pH and reserved.
m. The i fractions (precipitates) were rendered more soluble as follows: they were made 8M in urea, which was then gradually removed by dialysis against 0.01M sodium phosphate buffer pH 7. The resulting solutions were recombined with the respective s fractions.
n. Preparations A and B were tested for protein content with the Folin phenol reagent and for PRP antigenic activity by the assay described above. Both had PRP activity; B exceeded A by about 13-fold, as shown below:

Preparation	ng PRP equivalence/μg protein
CRM_{197}-PRP-S#2,A	0.038
CRM_{197}-PRP-S#2,B	0.50

o. Preparations A and B were tested for CRM antigenicity (activity as diphtheria toxoid (DT)) by inhibition of the binding of antibody to a sample of purified DT furnished by the Massachusetts Department of Public Health. Both had activity roughly equal to the DT on a weight basis; B exceeded A by about 4-fold, as shown below.

Inhibitor tested	Antibody bound, A_{400}	μg DT equivalence per μg protein
None	2.43	
DT, 0.5 μg/ml	2.56	
DT, 5 μg/ml	1.93	
DT, 50 μg/ml	0.96	
CRM_{197}-PRP-S#2,A,50 μg/ml	1.25	0.52
CRM_{197}-PRP-S#2,B,5 μg/ml	1.67	2.0

p. Preparations A and B were suspended in alum at 16 μg protein 1 ml, and three 0.5 ml injections were given to rabbits in the protocol described in Table 4 (except the animals were 8 weeks old at the onset and not primed by previous injections of diphtheria toxoid). The sera were tested for antibodies in the binding assay described in step o. Both A and B elicited antibodies to DT as well as to PRP, as shown in Table 6. Separate control experiments showed that similar rabbits housed in the same quarters did not display such increases in anti-DT antibody values in the absence of being injected with CRM_{197} preparations.

TABLE 6

Rabbit	Injected	Assay for antibody to	Antibody values at age 8 wk	9 wk	10 wk	11 wk
5	A	PRP, ng/ml	47	60	210	13,500
		DT, A400	0.136	0.168	1.28	3.81
6	A	PRP	21	25	19	420
		DT	0.072	0.049	0.262	3.23
7	A	PRP	<20	20	2000	10,500
		DT	0.155	0.134	0.155	0.676
3	B	PRP	<20	27	1600	4900
		DT	0.075	0.061	0.227	2.45
8	B	PRP	23	<20	2900	26,000
		DT	0.065	0.023	0.231	2.07

EXAMPLE: CONJUGATION OF VERY SMALL FRAGMENTS OF PRP TO DIPHTHERIA TOXIN, DIPHTHERIA TOXOID AND CRM_{197}

Generation of Very Small Fragments of PRP Containing Reducing End Groups

a. A 12 ml solution of PRP lot 20 was made 0.1M in HCl at 0° C. and sealed in a glass flask (0 minute).
b. The flask was transferred to a boiling-water bath for 4 minutes, then chilled in an ice water bath.
c. A small amount of resulting white colloid was removed by extraction with ether and the resulting clear solution was lyophilized.
d. Bio-Gel P10 (Bio Rad, Inc.) was equilibrated in 0.01M ammonium acetate and poured into a 1.5 cm diameter chromatographic column, giving a gel bed height of 98 cm.
e. The lyophilized material was rehydrated with 1.5 ml water and neutralyzed with NH_4OH. This solution was applied to the column and the elution was carried out.
f. Fragments eluting at Ve/Vo range of 2.0–2.4 were collected and designated fraction vs.
g. Steps a–f were repeated to double the supply of fraction vs.
h. The combined vs fractions were lyophilized, rehydrated to yield 4 ml of a solution containing a total of 47 μmoles of reducing sugar activity when assayed by the alkaline ferricyanide method standardized with D-ribose.

Preparation of Conjugates of PRP-vs Fragments to Native Diphtheria Toxin, Native Diphtheria Toxoid and CRM_{197}

The following proteins are used as carriers in the present example:

(1) DTx—purified diphtheria toxin, lot 1, obtained from the Massachusetts Public Health Biologic Laboratories. Partial detoxification is accomplished by the linking to PRPvs. Residual toxicity is removed by formalin treatment in the presence of lysine by the method of Pappenheimer et al., Immunochem. 9:891 (1972).
(2) DTd—conventional (formal) toxoid, lot DCP-27, also obtained from the Massachusetts laboratories.
(b 3) CRM_{197}—antigenically mutated version of the toxin protein, antigenically indistinguishable from toxin but non-toxic.

The conjugation method is as follows:

a. Protein, potassium phosphate buffer (pH 8.0 at 25° C.) and PRPvs were combined in glass centrifuge tubes in the manner set out below.

Solution	Protein	Buffer	PRPvs
(1)	30 mg DTx	0.24 μmol	20 μmol
(2)	30 mg DTd	0.24 μmol	20 μmol
(3)	10 mg CRM_{197}	0.08 μmol	6.7 μmol

b. The solutions were lyophilized, and the lyophiles were dissolved with $NaCNBH_3$ solution, 2% w/v in water as tabulated below.

Solution	2% $NaCNBH_3$
(1)	1.2 ml
(2)	1.2 ml
(3)	0.4 ml

c. The tubes were incubate at 37° C.
d. After 14 days, four volume-equivalents of saturated ammonium sulfate were added. These sus-

pensions were held 3 hours at 0° C., then centrifuged 20 minutes at 9000 G.

e. The precipitates were washed twice each with 10 ml of neutral 70% saturated ammonium sulfate.

f. The washed precipitates were dissolved with a minimal volume of 9.5M urea and dialyzed against 0.067M sodium phosphate buffer, pH 7.8.

Formalin Treatment of the Conjugates

The conjugates were further dialyzed against sodium phosphate buffer which also contained 0.025M lysine. (Small samples were reserved for toxicity testing prior to formalinization).

b. Formalin was added to a final concentration of 0.2% v/v.

c. After 17 days incubation at about 24° C. the solutions were extensively dialyzed against the sodium phosphate buffer.

d. Centrifugation was performed to remove small amounts of insoluble material.

Processing to Achieve Final Container Products

a. Antigen solutions (1)–(3) in isotonic sodium phosphate buffer were passed through 0.22-micron "Millex" filter units (Millipore Corp.) and injected into bottles containing sterile phosphate buffered saline.

b. The preparations were assayed for protein using the Lowry method.

c. Thimerosal was filtered and injected into the solution as 1/100 volume of a freshly made 1% w/v solution. Samples of 10 ml were taken for a sterility test. The bottles were attached to a manually operated sterile single use filling device (Multiple Additive Set, Travenol Laboratories). 2-ml glass vials were filled, stoppered, sealed, and immediately transferred to storage at 4° C.

Assays on Conjugate Preparations

a. Phosphate content of the protein fraction

PRP is composed of the repeating unit ribosyl-ribitol-phosphate. Thus colorimetric assay of phosphate in the fraction precipitable by 5% trichloracetic acid (TCA) is a sensitive index of the incorporation of PRP fragments into the protein.

Samples containing 100 μg protein were made 5% in TCA in a volume of 3 ml, held 20 minutes on ice, and centrifuged 15 minutes at 4° C. at G. The precipitates were washed with an additional 3 ml of 5% TCA, then with 5 ml ethanol. The washed precipitates were ashed to convert organic phosphate to inorganic phosphate (Pi), and the Pi was quantified by the method of Chen et al., Anal. Chem., 28:1756 (1956). The results were as follows:

Sample	nmol Pi/ μg protein	Implied average no. of PRP repeating units/protein
(1) DTx-PRPvs	0.11	6.8
(2) DTd-PRPvs	0.10	6.2
(3) CRM_{197}-PRPvs	0.10	6.2

b. Electrophoretic Analysis

Samples of the conjugated antigens were analyzed by mercaptoethanol-sodium dodecyl sulphate-polyacrylamide gel electrophoresis (ME-SDS-PAGE) in the same gel alongside the respective starting carrier protein preparations.

DTd-PRPvs, like the DTd, displayed a disperse band at MW 61,000 daltons. In contrast, DTx-PRPvs and CRM_{197}-PRPvs differed greatly from the starting proteins. The protein of these two conjugates collected either at the beginning of or in the stacking gel (4% acrylamide) or at the beginning of the separating gel (10% acrylamide). Thus, the conjugates appear to have been converted into macromolecular aggregates, presumably by cross-linking from the formalin treatment. DTd-PRPvs also contains some aggregated material.

c. PRP Antigen Equivalence Per Unit Protein

The capacity of the conjugates to bind anti-PRP antibody was determined by the inhibition of the binding of labeled PRP by human anti-PRP antiserum, calibrated with PRP lot 19. (Because protein-bound polymer fragments cannot be assumed to bind to antibody in a weight-equivalent fashion to the high molecular weight polymer, quantitative chemical composition cannot be inferred from these data.)

Sample	% Inhibition of ^{3}H-PRP bound	ng PRP equivalence/ μg protein
PBS control	(0)	—
PRP 19, 0.5 ng/ml	6.7	—
PRP 19, 5 ng/ml	32	—
PRP 19, 50 ng/ml	90	—
DTx-PRPvs, 5 μg protein/ml	24	0.5
DTd-PRPvs, 5 μg protein/ml	48	2.2
CRM_{197}-PRPvs, 5 μg protein/ml	38	1.4

d. Diphtheria Toxoid Antigenic Equivalence Per Unit Protein

Retention of the capacity of the preparations to react with anti-DTd antibody was determined by inhibition of an enzyme-linked immunosorbent assay (ELISA) in which purified DTd is attached to the assay tube (solid phase). Inhibition of antibody binding to the attached DTd is calibrated by the same DTd used in the fluid phase.

Sample	% Inhibition of Antibody Binding	μg DTd equivalence/ μg protein
PBS control	(0)	—
DTd, 5 μg/ml	24	—
DTd, 50 μg/ml	50	—
DTx-PRPvs, 50 μg protein/ml	46	0.68
DTd-PRPvs, 50 μg protein/ml	58	2.1
CRM_{197}-PRPvs, 50 μg protein/ml	26	0.11

e. Diphtheria Toxic Activity

Samples of the original DTx and the conjugate DTx-PRPvs before and after formalin treatment were titrated for toxic activity by injection into the skin of a non-immune adult rabbit. DTx at doses of 0.002 μg and 0.02 μg produced the expected dermal lesions. DTx-PRPvs prior to formalin treatment produced dose-dependent lesions such that 0.2 μg was approximately equal to 0.002 μg DTx (100-fold reduction in toxicity by the conjugation). After formalin treatment, lesions were not generated by doses as high as 2 μg (at least 1000-fold reduction relative to DTx). Doses up to 2 μg of conjugates DTd-PRPvs and CRM_{197}-PRPvs were tested similarly and generated no lesions.

f. Induction of Anti-PRP Antibody Responses in Weanling Rabbits, Measured by Radioantigen Binding

The antigens were mixed with an aluminum phosphate adjuvant (0.0125M Al, pH 6) such that a 0.5 ml dose contained 25 μg protein. Two rabbits (for each antigen) were given three weekly injections beginning at age 7 weeks; the rabbits had been injected with DTd alone at age 5 weeks, for a hypothetical "carrier priming" effect. All the animals (rabbits 1–6) had anti-PRP rises in an anamnestic pattern, with titers of at least 10 μg/ml after the third vaccination. Antigens CRM_{197}-PRPvs and DTd-PRPvs were also tested in pairs of rabbits that had not been "primed" with DTd. These (rabbits 7–10) produced strong anti-PRP reponses similar to those in the "primed" rabbits.

g. Induction of Anti-DTd Antibody Response in Weanling Rabbits, Measured by ELISA

The anti-DTd antibody responses in the same "unprimed" rabbits (7–10) of the preceding subsection are as follows: Rises were roughly 10-fold after the second injection and another 2- to 5-fold after the third.

h. Sterility of the Sample Preparations

The samples were found to be sterile as determined using Fluid Thioglycollate (BBL cat. no. 11260, lot D4D LKL) as the growth medium.

EXAMPLE: USE OF PRP FRAGMENTS CONJUGATED TO DIPHTHERIA TOXOID AND CRM_{197} AS VACCINES IN YOUNG HUMANS

Two groups of 8 children in the age range of 1 to 2 years old (and specifically exempting children receiving routine vaccination with diphtheria toxoid protein at age 18 months) were given primary and secondary vaccinations as follows: Group I received injections of CRM_{197}-PRPvs preparation as described in the preceding section (25 g protein in saline, subcutaneously); Group II received injections of DTd-PRPvs, preparation as described in the preceding section (25 μg protein in saline, subcutaneously).

In the first visit, pre-vaccination blood specimens were taken; the child was vaccinated, then observed for 20 minutes for any sign of an anaphylactic reaction. In the second visit the procedure of the first visit was repeated. In the third visit, a post-secondary blood specimen was taken. Two of the children, one from each group, after consultation with the parents, were given a third vaccination to try to raise the antibody against PRP to protective levels. The interval between vaccinations was 1±¼ month.

Group III consisted of children about 18 months old receiving a vaccine simultaneously with diphtheria toxoid protein in a separate site. This group contained 2 children; one received the CRM_{197}-PRPvs vaccine, the other received the DTd-PRPvs vaccine.

Symptoms were recorded for four successive days, with measurements of temperature, notation of behavioral indications of systemic illness and observations of inflammation at the injection site. These symptoms are summarized in Table 7.

TABLE 7

ADVERSE REACTIONS TO PRP-VS CONJUGATES TO CRM_{197} AND FORMAL DIPHTHERIA TOXOID

Vaccine	Symptom	Injection		
		Primary	Secondary	Tertiary
CRM_{197}-PRPvs	Fever	1/8	0/8	0/1
	Unusual behavior	0/8	0/8	0/1
	Local inflammation	1/9*	2/9	0/1
	Local pain	1/9*	1/9	0/1

TABLE 7-continued

ADVERSE REACTIONS TO PRP-VS CONJUGATES TO CRM_{197} AND FORMAL DIPHTHERIA TOXOID

Vaccine	Symptom	Injection		
		Primary	Secondary	Tertiary
DTd-PRPvs	Fever	0/8	0/8	0/1
	Unusual behavior	0/8	0/8	0/1
	Local inflammation	1/9*	0/9	0/1
	Local pain	1/9	1/9	0/1

*Includes one child who received diphtheral toxoid protein simultaneously in a separate site. No local symptoms were found. Systemic symptoms are not noted since these could not be distinguished from an effect of the diphtheria toxoid protein vaccine.

After CRM_{197}-PRPvs vaccination, one child had mild fever (99.8° F.) on the evening of primary vaccination; there was an instance of mild local inflammation once each after a primary, a secondary, and the one tertiary vaccination. After DTd-PRPvs there was an instance of local inflammation after one primary and one secondary vaccination. The administration of the vaccines was otherwise apparently free of adverse reactions.

Serum Antibody Reponses

Antibodies to PRP as well as IgG antibodies to diphtheria toxoid were determined. After vaccination with CRM_{197}-PRPvs a consistent anti-PRP response pattern was seen. See Table 8. There was a distinct rise after the primary injection, usually an even larger rise after the secondary injection, and a large rise after the one tertiary. The final titers greatly exceeded those that have been produced by vaccination with PRP alone and greatly exceeded the accepted estimated protective minimal level of 0.15 μg/ml. The enhanced response was particularly evident in the four children under 18 months of age, where the response to PRP alone is generally inadequate for protection, and the geometric mean of the final titers in these four (8.4 μg/ml) is 175 times that found after vaccination of children 12–17 months old with PRP vaccine alone. The child receiving the primary vaccination simultaneously with diphtheria toxoid protein vaccine also had an excellent response.

IgG antibodies to diphtheria toxoid increased in 6 of 8 children (as well as in the 9th, who also received diphtheria toxoid as part of the treatment). The antibody levels often increased so greatly that the dilution of post-vaccination serum used (1/1000) was insufficient to show the full extent of the rise.

After vaccination with DTd-PRPvs anti-PRP responses generally increased after both primary and secondary vaccination. (See Table 9). However, there were two children (12 and 14 month old) in whom no response was detected; and one child did not approach the protective level until given a third injection. The child receiving the primary vaccination simultaneously with diphtheria toxoid protein had an excellent response. Rises in IgG antibody to the diphtheria component were found in all children.

TABLE 8

ANTIBODY RESPONSE TO CRM_{197}-PRPvs

Subject	Age at primary vaccination	Serum sample	Serum antibody, μg/ml	
			anti-PRP	IgG anti-DTd
1	12 mo	pre-vac	2.0	1.1
		post-1	4.5	>10

TABLE 8-continued

ANTIBODY RESPONSE TO CRM_{197}-PRPvs

Subject	Age at primary vaccination	Serum sample	Serum antibody, μg/ml anti-PRP	IgG anti-DTd
		post-2	18	>10
2	13 mo	pre-vac	<0.006	0.38
		post-1	0.040	1.7
		post-2	0.35	2.2
		post-3	4.8	1.9
3	14 mo	pre-vac	<0.020	4.5
		post-1	0.12	3.3
		post-2	2.0	4.3
4	16 mo	pre-vac	0.025	0.06
		post-1	0.92	5.7
		post-2	29	9.1
5	27 mo	pre-vac	0.025	3.0
		post-1	10	>10
		post-2	58	>10
6	29 mo	pre-vac	0.13	6.1
		post-1	22	6.9
		post-2	180	7.4
7	30 mo	pre-vac	2.2	6.5
		post-1	28	>10
		post-2	50	>10
8	30 mo	pre-vac	1.3	4.8
		post-1	6.5	10
		post-2	78	10
9	18 mo*	pre-vac	0.34	3.1
		post-1	1.4	>10
		post-2	8.2	>10

*First injection of CRM_{197}-PRPvs given simultaneously with diphtheria toxoid protein vaccine in a separate site.

TABLE 9

ANTIBODY RESPONSE TO DTd-PRPvs

Subject	Age at primary vaccination	Serum sample	Serum antibody, μg/ml anti-PRP	IgG anti-DTd
1	12 mo	pre-vac	<0.020	0.060
		post-1	<0.020	10
		post-2	<0.020	10
2	12 mo	pre-vac	0.055	0.03
		post-1	0.080	3.1
		post-2	1.8	10
3	13 mo	pre-vac	<0.006	1.1
		post-1	<0.006	10
		post-2	0.023	10
		post-3	0.120	10
4	14 mo	pre-vac	<0.020	3.0
		post-1	<0.020	5.1
		post-2	<0.020	3.8
5	19 mo	pre-vac	0.060	8.0
		post-1	0.12	10
		post-2	0.76	10
6	26 mo	pre-vac	<0.020	6.9
		post-1	0.060	10
		post-2	0.94	10
7	27 mo	pre-vac	1.4	6.1
		post-1	7.4	10
		post-2	21	10
8	28 mo	pre-vac	<0.020	8.7
		post-1	0.63	10
		post-2	8.0	10
9	18 mo*	pre-vac	1.9	0.11
		post-1	2.9	10
		post-2	11	10

*First injection of DTd-PRPvs given simultaneously with diphtheria toxoid protein vaccine in a separate site.

This example shows that injections of conjugates of the *H. influenzae* b capsular polymer fragment to diphtheria toxoid and CRM_{197} is apparently harmless. CRM_{197}-PRPvs vaccination gave a clear indication of an enhancement of the anti-PRP response by the carrier effect - appreciated not only by the high titers but by the rises after secondary vaccination.

DTd-PRPvs had a less impressive enhancement. A likely explanation is that while CRM_{197}-PRPvs is a multimolecular aggregate, DTd-PRPvs is present mainly in unimolecular form similar to the original toxoid.

EXAMPLE: CONJUGATION OF CAPSULAR POLYMER FRAGMENTS OF STREPTOCOCCUS PNEUMONIAE TO CRM_{197}

Several other bacteria resemble *H. influenzae* b in that they cause sepsis and meningitis, particularly in infants; they have polymer capsules, antibodies to which are protective; and their capsular polymers are immunogenic in mature humans but not in infants. An important example is *Streptococcus pneumoniae* (SP) serotype 6. It causes not only the life-threatening infections mentioned above but also is a highly prevalent cause of otitis media in children. (Gray et al., J. Infect. Dis. 142: 923–33, 1980).

The approach described for PRP is also applicable to any capsular polymer in which reducing groups can be generated by selective hydrolysis with retention of antigenic specificity. In the following non-limiting example, capsular polymer fragments were made from the Sp. 6 capsular polymer by selective acid hydrolysis and were conjugated to CRM_{197}. The product retained antigenic specificity for both the Sp capsular polymer and the CRM_{197} component.

Generation of Reducing Fragments From Capsular Polymers (CP)

1. A sample of the CP of Sp. 6 (Danish type 6A, Eli Lilly Co.) was assayed for total hexose by the phenol-sulfuric acid method standardized with D-glucose and for reducing activity by the alkaline ferricyamide method also standardized with D-glucose.
2. Pyrex tube received 3.3 mg Sp. 6 CP dissolved with 0.66 ml water. The sample was chilled to 0° C., 0.073 ml of 0.1N HCl were added, and the tube was sealed.
3. The tube was immersed 10 minutes in a boiling water bath, then rechilled to 0° C. A small sample was assayed for reducing activity as described in step 1:

CP	Time heated at 100° C.	Total hexose/ reducing hexose
Sp. 6	0 minutes	>350
	10 minutes	6.5

4. The hydrolyzed preparation (minus the 2% used for assay) was lyophilized. The dried material was dissolved with 0,1 ml water, transferred to microcentrifuge tube, and lyophilized again.

Conjugation to CRM_{197}

1. To the re-dried hydrolysate was added 0.004 ml of 2M potassium phosphate buffer pH 8 and 1 mg of CRM_{197} dissolved in 0.2 ml of 0.01M sodium phosphate buffer pH 7. The resulting mixture was lyophilized and resuspended with 0.05 ml water (estimated total volume 0.063 ml).
2. To the tube was added 0.007 ml of sodium cyanoborohydride at 200 mg/ml, and the preparation was incubated 18 days at 37° C.
3. 0.6 ml 80% saturated ammonium sulfate (SAS) was added.

4. The tube was incubated 1 hour at 0° C. and centrifuged 15 minutes at 8000 G; the supernate was removed.
5. The precipitate was washed by suspension in 0.6 ml of 80% SAS buffered at pH 8 with 0.01M sodium phosphate, followed by centrifugation 15 minutes at 8000 G.
6. The precipitate was suspended with 0.02 ml of 0.5M Na_2HPO_4 and 0.2 ml 9.5M urea.
7. 1 ml SAS was added, the precipitate was isolated as in step 4 and suspended in urea at about 8M as in step 6.
8. The suspension was centrifuged 15 minutes at 8000 G.
9. The supernate was separated and dialyzed against 0.01M sodium phosphate buffer pH 7 at 4° C.
10. The dialyzed preparations, designated CRM_{197}-Sp.6 was assayed for the following:
protein by the Folin phenol reaction;
Sp antigenicity by inhibition of binding of antibody to radiolabeled Sp CP (as described for PRP in Table 3);
CRM_{197} antigenicity by the inhibition of antibody binding to diphtheria toxoid (DT) (as described in step o of the description of CRM_{197}-PRP-S#2); and
anti-CP immunogenicity by inhibition of the binding of antibody to diphtheria toxoid (DT) (as described in step p of the description of CRM_{197}-PRP-S#2). See Table 7.

Preparation	ng CP equivalance/ μg Protein	μg DT equivilance/ μg protein
CRM_{197}Sp. 6	0.4	0.36

TABLE 10

ANTI-CP IMUNOGENIC RESPONSE OF WEANLING RABBITS WITH CONTROLS AND CONJUGATES OF *STREPTOCOCCUS PNEUMONIAE* SEROTYPE 6 FRAGMENTS OF CRM_{197}

Rabbit	Vaccinated With*	Percent ^{14}C-CP Bound in Samples at age** 6 wk	8 wk	10 wk	11 wk
1	Sp 6 CP, 25 μg	6	6	7	7
2	"	6	13	13	11
3	Sp 6 bacteria 25 μg	4	10	12	16
4	"	8	12	22	21
5	CRM_{197} Sp 6, 25 μg	4	6	30	49
6	"	4	8	30	54

*Injected subcutaneously just prior to taking serum samples. Serum samples were taken at age 6, 8 and 10 weeks.
**25 μl serum incubated with 2 nCi ^{14}C-labelled CP.

EXAMPLE; CONJUGATION OF PRP FRAGMENTS PRODUCED BY OXIDATIVE CLEAVAGE TO CRM_{197}

In this example, the final conjugate comprises two components; fragments of PRP of reasonably well defined chain length covalently linked to the non-toxic, immunogenic diptheria toxin CRM_{197}. In this example, conditions of the periodate oxidation and isolation by ultrafiltration govern the chain length of the capsular polymer (PRP) fragments.

The conjugate is constructed with capsular polymer fragments having an aldehyde group at both ends of the fragment. Thus, each fragment can be covalently linked to CRM at both ends, presumably at lysine residues of CRM. The structure of the product can, therefore, be considered a "lattice."

The composition and presumably the structure of the conjugate can be altered by changing the concentration of the two components in the conjugating chemical reaction.

The PRP used to produce the PRP fragments for the PRP-CRM conjugate vaccine had the following specifications:

Protein content	<1.0%
Nucleic acids content	≦1.0%
Endotoxin (LAL)	<1.0 EU/μg PRP
Molecular size (Kd)	<0.3 (Sepharose CL-4B)
	<0.6 (Sepharose CL-2B)

Production Methods for PRP Fragments

a. A solution of PRP (5-7 mg/ml) was cooled to 4° C. and 2M phosphate buffer pH 7.0 was added to make the final concentration 0.2M phosphate.
b. Sodium metaperiodate (0.2-0.3 moles IO_4/mole PRP) was added with rapid mixing, and the solution was incubated at 4° C. overnight in the dark.
c. The reaction solution was ultrafiltered using a H1P30 hollow fiber (Amicon, 30,000 Mw cut-off). The retentate was washed 4 times with 250 ml of saline. The filtrates were combined and ultrafiltered using a H1P10 hollow fiber (Amicon, 10,000 Mw cut-off). The retentate was washed 4 times with 250 ml of saline. The retentate was then concentrated to >35 mg of PRP/ml.
d. The retentate was analyzed for ribose by orcinol assay and reducing groups by alkaline ferricyanide assay. A small aliquot of the retentate was analyzed on a Biogel P-100 column (0.7×25 cm) using 0.15M saline as elutant and analyzing each of the fractions by orcinol and alkaline ferricyanide assay. The analysis showed that the retentate material was composed of oligosaccharides having a DP between 15 and 30 with a weight average DP of about 20.

For capsular polymer fragments prepared by oxidative cleavage chain length (DP) is defined as (ribose units/reducing groups)×2.

Two batches of periodate oxidized PRP were fractionated as in part d and were characterized as follows:

SACCHARIDE CHAIN LENGTH

Fraction No.	DP of Fraction BATCH #2	DP of Fraction BATCH #3	% of Total BATCH #2	% of Total BATCH #3
14	36.5	41.0	2.7	5.9
15	24.1	32.5	11.0	9.4
16	25.3	24.5	13.2	11.8
17	25.4	22.8	14.4	14.3
18	23.2	20.2	13.6	14.6
19	20.2	19.0	11.9	14.2
20	20.4	17.7	9.3	12.4
21	16.1	16.0	6.7	10.0
22	11.7	12.3	7.2	7.1

CRM-PRP Conjugation

a. CRM protein was dissolved in 0.2M sodium phosphate buffer (pH 7.0) at a final concentration of 10 mg/ml.

b. Lyophilized oligosaccharide was reconstituted in distilled water, an appropriate quantity (average DP of 20) was added to the CRM protein, and the solution was mixed.

c. Sodium cyanoborohydride (0.5 g/ml) (10× molar excess) was added, and the solution was mixed and incubated at 37° C. for 3 days.

d. Sodium borohydride solution (100× the reducing groups) was added, and the solution was allowed to stand at room temperature for 2 hours.

e. The conjugate was diluted 10× with 6M urea to dissolve any precipitate, and the solution was ultrafiltered using an Amicon stirred cell with a YM-30 (30,000 Mw cut-off) membrane.

f. The solution was repeatedly ultrafiltered using sterile saline until the filtrate was negative for pentose and cyanide ion.

Properties of the Final Conjugates

Table 11 presents various characteristics of the PRP-CRM_{197} conjugates from various batches of exidized PRP and lots resulting from several conjugation runs:

TABLE 11

	PRODUCTS OF CRM-PRP CONJUGATION			
VACCINE LOT NO.	PRP SACCHARIDE BATCH	RATIO OF PRP/CRM IN REACTION MIXTURE (μg/μg)	RATIO OF PRP/CRM IN FINAL CONJUGATE (μg/μg)	Kd* (SEPHAROSE CL-4B)
5	#2	1.0	0.25	0.27
6	#2	2.0	0.62	0.31
7	#3	1.0	0.38	0.36
8	#3	2.0	0.57	0.44
9	#6	1.0	0.60	0.48
10	#6	2.0	0.42	0.48
11	#7	1.0	0.27	0.30
12	#7	2.0	0.42	0.48

*Kd at which 50% of the material (protein) elutes.

Bottling

a. The CRM-PRP conjugate is sterile filtered through a 0.8 and then a 0.22 micron filter into a tared sterile container.

b. A sample is removed aseptically and analyzed for protein by Lowry assay.

c. The volume of filtered material is determined by weighing the container and a final volume calculated to give 50 μg of protein per ml.

d. An amount of 1% Thimerosal in sterile saline is added through a sterile 0.22 micron filter to give 0.01% Thimerosal in the final solution.

e. The vaccine is brought to the final volume with sterile saline filtered through a 0.22 micron filter.

f. The solution is mixed and 5.5 ml aliquoted into sterile, pyrogen-free 10 ml vials (Wheaton, type 1 glass), which are stopped (butyl gray rubber, Wheaton), sealed, and stored at 2°-8° C.

	FINAL DOSAGE FORMULATIONS*		
Vaccine Lot No.	Protein (μg/ml)	PRP (μg/ml)	Kd**
5	50	12.5	0.27
6	50	31.0	0.31
7	50	19.0	0.36
8	50	28.5	0.44
9	50	30.0	0.48
10	50	21.0	0.48
11	50	13.5	0.30
12	50	21.0	0.48

*All formulations are made up in 0.9% NaCl and 0.01% Thimerosal.
**Kd at which 50% of the material (protein) elutes.

Testing In Vitro Antigenicity

Serial dilutions of vaccine in PBS 0,05% Tween were added in duplicate to wells of microtiter plates precoated with diphtheria toxoid, A pooled high-titered polyclonal human diphtheria antitoxin serum or a human polyclonal anti-PRP serum was then added to the wells and the plates incubated at 20° C. for 24 hours. Antibody binding was determined by subsequent incubation of the wells with enzyme-labelled anti-human immunoglobulin followed by incubation for 60 minutes with the enzyme substrate and quantitation of the optical density. The results are presented in Table 12.

TABLE 12

In Vitro Antigenicity of PRP-CRM Conjugate
Inhibition of Binding of Human Polyclonal
Anti-Diphtheria Toxoid and Anti-PRP Antibodies

	EQUIVALENT OF INHIBITOR ON BINDING	
VACCINE	ANTI-PRP	ANTI-DIP TOXOID
PRP	(1)	—
DT-Mass*	—	(100)
Lot #5	1.5	87.0
Lot #6	1.1	57.0
Lot #7	3.3	9.3
Lot #8	2.9	8.0

*Diphtheria toxoid supplied by the Massachusetts Public Health Biologic Laboratories, lot Dcp 27.

The data in Table 12 indicate that the various lots of vaccine retain in vitro antigenicity: they can compete with polyclonal antibodies to PRP and to diphtheria toxoid. The data also indicate that the PRP antigen is relatively exposed while the diphtheria toxoid epitopes are less well and variably exposed.

Immunogenicity in Animals

a. Rabbits

Table 13 summarizes three experiments in which young rabbits were vaccinated at week 0, 1, and 2 with 25 μg of vaccine. All vaccines were immunogenic and gave boostable anti-PRP responses as determined by ELISA assay. Moderate anti-PRP responses were seen

even after a single injection of 25 μg of conjugate vaccine.

b. Mice

The results obtained with vaccination of young Swiss Webster mice again produced a boostable anti-PRP response (data not shown)

TABLE 13

ANTI-PRP RESPONSES IN WEANLING RABBITS TO VARIOUS LOTS OF PRP-CRM CONJUGATES

VACCINE LOT NO.	WEEK 0 μg/ml (GMT)	WEEK 1 μg/ml (GMT)	WEEK 3 μg/ml (GMT)
5	0.1	0.23	7.56
6	0.1	0.38	2.86
7	0.2	0.86	9.70
8	0.1	0.87	7.61
9	0.1	0.36	3.88
10	0.1	0.92	1.84
11	0.1	0.21	5.01
12	0.1	0.31	2.33

Immunogenicity in Human Infants

Infant subjects were healthy and had no prior immunization with Hib vaccine nor history of serious adverse reaction to vaccines. Beginning at the ages noted in Table 14 (18, 7 and 2 mo., respectively), they were bled, given a 25 μg subcutaneous primary injection of PRP-CRM conjugate, observed at least 20 minutes and released to their parents for observation and recording of possible local and systemic adverse reactions.

For the 7-mo. and 2-mo. groups, after lapse of the times indicated in Table 14, the process was repeated for a secondary immunization with the same vaccine. After another lapse of time (see Table 14), they were again bled for determination of the secondary response.

As can be seen from Table 14, single injections were effective in raising antibodies in age groups 18 and 7 months, while in 2 month old infants a modest increase in antibody was observed (despite the expected decline in maternal IgG antibodies). In all of the infants, adequate levels of anti-PRP antibody were observed 1-2 months after a second immunization. The response observed in 6 month old infants after two immunizations was sufficient to elicit protective levels of antibodies.

TABLE 14

ANTI-PRP ANTIBODY RESPONSE to 25 μg VACCINE

Age (mos.) at Vaccination	No. of Children	Antibody, μg/ml* Pre	Post 1	Post 2
18-23	84	0.40 1 mo.	6.53	
7-15	88	0.15 1-2 mo.	4.54 1-2 mo.	18.9
2-6	30	0.17 2 mo.	0.25 2 mo.	1.23

*Geometric Mean Titer.

EXAMPLE: COUPLING OF PERIODATE-OXIDIZED PNEUMOCOCCAL POLYSACCHARIDES TO DIPHTHERIA TOXOID

Table 15 presents a summary of several experiments in which periodate-oxidized pneumococcal polysaccharides were coupled to diphtheria toxoid. The pneumococcal capsular polysaccharides (PnPS) of the types indicated in the table were reacted for 90 minutes at 37° C. with the indicated amount of sodium periodate, and then recovered by filtration on a Centricon-10 ultrafiltration device (Amicon).

The oxidized PS were reacted with 4mg of purified toxoid lot Dcp 27 and 10 mg of $NaCNBH_3$ in a total volume of 0.75 ml for 3 days at 37° C. at pH 8. The protein fraction was recovered by precipitation and washing with 90% saturated ammonium sulfate. The Pn PS antigenic equivalence was assayed by radioantigen binding inhibition using human antiserum to Pn PS and ^{14}C labeled Pn PS.

TABLE 15

Coupling of Periodate-Oxidized Pneumococcal Polysaccharides (Pn PS) to Diphtheria Toxoid by Reductive Amination

Pn PS type	μmol IO_4 reacted with 10 mg PS	Pn PS antigenic activity recovered in protein fraction after coupling reaction (μg PS/μg protein)
3	50	0.1
6A	4	0.2
12	4	0.1
14	6	0.1
23	4	0.1

Having described the invention with particular reference to certain embodiments, it will be obvious to those skilled in the art to which the invention pertains after understanding the invention, that various changes and modifications may be made without departing from the spirit and scope of the invention as defined by the appended claims.

We claim:

1. An immunogenic conjugate comprising the reductive amination product of an intact capsular polymer of the bacterial pathogen *Streptococcus pneumoniae* having at least two carbonyl groups and a bacterial toxin or toxoid, said conjugate comprising a cross-linked conjugate in which there is a direct covalent linkage between the capsular polymer and the toxin or toxoid.

2. The immunogenic conjugate of claim 1, in which the bacterial pathogen is *Streptococcus pneumoniae* serotype 3.

3. The immunogenic conjugate of claim 1, in which the bacterial pathogen is *Streptococcus pneumoniae* serotype 12.

4. The immunogenic conjugate of claim 1, in which the bacterial pathogen is *Streptococcus pneumoniae* serotype 14.

5. The immunogenic conjugate of claim 1, in which the bacterial pathogen is *Streptococcus pneumoniae* serotype 23.

6. The immunogenic conjugate of claim 1, in which the toxin or toxoid is diphtheria toxin or toxoid.

7. The immunogenic conjugate of claim 6, in which the toxoid is CRM_{197}.

* * * * *

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:

Porter W. Anderson and
Ronald J. Eby

Group Art Unit: 1813

Examiner: K. Kim

Serial No.: 07/819,305

Filed: January 9, 1992

For: IMMUNOGENIC CONJUGATES OF STREPTOCOCCUS PNEUMONIAE CAPSULAR POLYMER AND TOXIN OR TOXOID (as amended herein)

Attorney Docket: 3117-081

TERMINAL DISCLAIMER UNDER 37 C.F.R. §1.321(b) AND CERTIFICATE UNDER 37 C.F.R. §3.73(b)

Honorable Commissioner of Patents and Trademark
Washington, D.C. 20231

S I R :

Your petitioner, Jane A. Youngers, residing at

22 Hollywood Crescent, Rochester, New York 14618

_____, represents that she is Director of Research and Project Administration of The University of Rochester, assignee of the entire right, title, and interest in patent application Serial No. 07/819,305 filed January 9, 1992, for IMMUNOGENIC CONJUGATES OF STREPTOCOCCUS PNEUMONIAE CAPSULAR POLYMER AND TOXIN OR TOXOID. The assignment was recorded at Reel 5108, Frame 0081. Your petitioner, in her capacity as Director of Research and Project Administration of The University of Rochester hereby disclaims the terminal part of any patent granted on the above-identified application which could extend beyond the expiration date of the following:

application Serial No. [illegible].048, filed July 5, 1983 for "IMMU[illegible]GENIC CONJUGATES", which is a Continuation-in-Part of U.S. Patent Application Serial No. 298,102, filed August 31, 1981 (abandoned), the entire right, title, and interest in which is assigned to The University of Rochester. This assignment was recorded at Reel 4418, Frame 4580;

2. U.S. Patent No. 4,761,283, which issued on August 2, 1988 based on application Serial No. 845,731, filed March 28, 1986 for "IMMUNOGENIC CONJUGATES," which is a Continuation of Application Serial No. 511,048, currently Patent No. 4,673,574, the entire right, title, and interest in which is assigned to The University of Rochester. This assignment was recorded at Reel 4418, Frame 4580;

3. U.S. Patent No. 4,902,506, which issued on February 20, 1990 based on application Serial No. 859,975, filed May 5, 1986, for "IMMUNOGENIC CONJUGATES," which is a Continuation-in-Part of Application Serial No. 511,048, currently Patent No. 4,673,574. The entire right, title and interest in Application Serial No. 859,975 was originally assigned from the inventors to Praxis Biologics, Inc. This assignment was recorded at Reel 4658, Frame 0682; and then the entire right, title, and interest in which was then assigned to The University of Rochester. This assignment was recorded at Reel 5108, Frame 0081; and

4. U.S. Patent No. 5,097,020, which issued on March 17, 1992 based on application Serial No. 423,081, filed October 18, 1989 for "IMMUNOGENIC CONJUGATES", which is a Continuation of Application Serial No. 859,975, currently Patent No. 4,902,506, the entire right, title, and interest in which is assigned to The

University of Rochester [as set forth in (3) above]. This assignment was recorded at Reel 5108, Frame 0081.

Your Petitioner further agrees that any patent granted on application Serial No. 07/819,305 shall be enforceable only for and during such period that the legal title thereto shall be the same as the legal title to the following:

1. U.S. Patent No. 4,673,574 granted on June 16, 1987 based on application Serial No. 511,048;
2. U.S. Patent No. 4,761,283 granted on August 2, 1988 based on application Serial No. 845,731;
3. U.S. Patent No. 4,902,506 granted on February 20, 1990 based on application Serial No. 859,975; and
4. U.S. Patent No. 5,097,020 granted on March 17, 1992 based on application Serial No. 423,081;

this agreement to run with any patent granted on application Serial No. 07/819,305 and to be binding upon the grantee, its successors or assigns.

Petitioner does not disclaim any terminal part of any patent granted on said above-identified application prior to the expiration date of the full statutory term of U.S. Patent Nos. 4,673,574; 4,761,283; 4,902,506; and/or 5,097,020, as presently shortened by any terminal disclaimer, in the event that U.S. Patent Nos. 4,673,574; 4,761,283; 4,902,506; and/or 5,097,020 later: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid, is statutorily disclaimed in whole or terminally disclaimed under 37 C.F.R. 1.321(a), has all claims cancelled by a reexamination certificate, or is

any terminal disclaim[illegible] except for the separation of legal ti[illegible]tated above.

The undersigned has reviewed all the documents in the chain of title of the patent application identified above and, to the best of undersigned's knowledge and belief, title is in the assignee identified above.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 29 Dec. 93

Jane A. Youngers
Director, Research and Project Administration
THE UNIVERSITY OF ROCHESTER

Signed at Rochester (City) Newyork USA (Country)

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MAINTENANCE FEE STATEMENT

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 10, "status" below.

If a maintenance fee payment is defective, the reason is indicated by code in column 10, "status" below. An explanation of the codes appears on the reverse of the Maintenance Fee Statement. TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

ITM NBR	PATENT NUMBER	FEE CDE	FEE AMOUNT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT
1	5,360,897	183	1050	----	07/819,305	11/01/94	01/09/92	04	NO	PAID

If the "status" column for a patent number listed above does not indicate "PAID" a code or an asterisk (*) will appear in the "status" column. Where an asterisk (*) appears, the codes are set out below by the related item number. An explanation of the codes indicated in the "status" column and as set out below by the related item number appears on the reverse of the maintenance fee statement.

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1	3117-061

DIRECT THE RESPONSE TOGETHER WITH ANY QUESTIONS ABOUT THIS NOTICE TO:
COMMISSIONER OF PATENTS AND TRADEMARKS, BOX M, FEE, WASHINGTON, DC 20231

Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein)

Prevnar™

CI 6044-1

Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein)

Prevnar™

℞ only

For Intramuscular Injection Only

DESCRIPTION

Prevnar™, Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein), is a sterile solution of saccharides of the capsular antigens of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F individually conjugated to diphtheria CRM_{197} protein. Each serotype is grown in soy peptone broth. The individual polysaccharides are purified through centrifugation, precipitation, ultrafiltration, and column chromatography. The polysaccharides are chemically activated to make saccharides which are directly conjugated to the protein carrier CRM_{197} to form the glycoconjugate. This is effected by reductive amination. CRM_{197} is a nontoxic variant of diphtheria toxin isolated from cultures of *Corynebacterium diphtheriae* strain C7 (β197) grown in a casamino acids and yeast extract-based medium. CRM_{197} is purified through ultrafiltration, ammonium sulfate precipitation, and ion-exchange chromatography. The individual glycoconjugates are purified by ultrafiltration and column chromatography and are analyzed for saccharide to protein ratios, molecular size, free saccharide, and free protein.

The individual glycoconjugates are compounded to formulate the vaccine, Prevnar™. Potency of the formulated vaccine is determined by quantification of each of the saccharide antigens, and by the saccharide to protein ratios in the individual glycoconjugates.

Prevnar™ is manufactured as a liquid preparation. Each 0.5 mL dose is formulated to contain: 2 µg of each saccharide for serotypes 4, 9V, 14, 18C, 19F, and 23F, and 4 µg of serotype 6B per dose (16 µg total saccharide); approximately 20 µg of CRM_{197} carrier protein; and 0.125 mg of aluminum per 0.5 mL dose as aluminum phosphate adjuvant.

After shaking, the vaccine is a homogeneous, white suspension.

CLINICAL PHARMACOLOGY

S. pneumoniae is an important cause of morbidity and mortality in persons of all ages worldwide. The organism causes invasive infections, such as bacteremia and meningitis, as well as pneumonia and upper respiratory tract infections including otitis media and sinusitis. In children older than 1 month, *S. pneumoniae* is the most common cause of invasive disease.[1] Data from community-based studies performed between 1986 and 1995, indicate that the overall annual incidence of invasive pneumococcal disease in the United States is an estimated 10 to 30 cases per 100,000 persons, with the highest risk in children aged less than or equal to 2 years of age (140 to 160 cases per 100,000 persons).[2,3,4,5,6] Children in group child care have an increased risk for invasive pneumococcal disease.[7,8] Immunocompromised individuals with neutropenia, asplenia, sickle cell disease, disorders of complement and humoral immunity, human immunodeficiency virus (HIV) infections or chronic underlying disease are also at increased risk for invasive pneumococcal disease.[9] *S. pneumoniae* is the most common cause of bacterial meningitis in the United States.[1] The annual incidence of pneumococcal meningitis in children between 1 to 23 months of age is approximately 7 cases per 100,000 persons.[1] Pneumococcal meningitis in childhood has been associated with 8% mortality and may result in neurological sequelae (25%) and hearing loss (32%) in survivors.[9]

S. pneumoniae is an important cause of acute otitis media, identified in 20 to 40% of middle ear fluid cultures.[10,11] The seven serotypes account for approximately 60% of acute otitis media due to *S. pneumoniae* (12-24% of all acute otitis media).[12] The exact contribution of *S. pneumoniae* to childhood pneumonia is unknown, as it is often not possible to identify the causative organisms. In studies of children less than 5 years of age with community-acquired pneumonia, where diagnosis was attempted using serological methods, antigen testing, or culture data, 30% of cases were classified as bacterial pneumonia, and 70% of these (21% of total community-acquired pneumonia) were found to be due to *S. pneumoniae*.[13,14]

In the past decade the proportion of *S. pneumoniae* isolates resistant to antibiotics has been on the rise in the United States and worldwide. In a multi-center US surveillance study, the prevalence of penicillin and cephalosporin-nonsusceptible (intermediate or high level resistance) invasive disease isolates from children was 21% (range < 5% to 38% among centers), and 9.3% (range 0-18%), respectively. Over the 3-year surveillance period (1993-1996), there was a 50% increase in penicillin-nonsusceptible *S. pneumoniae* (PNSP) strains and a three-fold rise in cephalosporin-nonsusceptible strains.[8] Although generally less common than PNSP, pneumococci resistant to macrolides and trimethoprin-sulfazoxole have also been observed.[4] Day care attendance, a history of ear infection, and a recent history of antibiotic exposure, have also been associated with invasive infections with PNSP in children 2 months to 59 months of age.[7,8] There has been no difference in mortality associated with PNSP strains.[15] However, the American Academy of Pediatrics (AAP) revised the antibiotic treatment guidelines in 1997 in response to the increased prevalence of antibiotic-resistant pneumococci.[16]

Approximately 90 serotypes of *S. pneumoniae* have been identified based on antigenic differences in their capsular polysaccharides. The distribution of serotypes responsible for disease differ with age and geographic location.[16]

Serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F have been responsible for approximately 80% of invasive pneumococcal disease in children < 6 years of age in the United States.[17] These 7 serotypes also accounted for 74% of PNSP and 100% of pneumococci with high level penicillin resistance isolated from children < 6 years with invasive disease during a 1993-1994 surveillance by the Centers for Disease Control.[17]

Results of Clinical Evaluations

Efficacy

Efficacy was assessed in a randomized, double-blinded clinical trial in a multiethnic population at Northern California Kaiser Permanente (NCKP), beginning in October 1995, in which 37,816 infants were randomized to receive either Prevnar™ or a control vaccine (an investigational meningococcal group C conjugate vaccine [MnCC]) at 2, 4, 6, and 12-15 months of age. Prevnar™ was administered to 18,906 children and the control vaccine to 18,910 children. Routinely recommended vaccines were also administered which changed during the trial to reflect changing AAP and Advisory Committee on Immunization Practices (ACIP) recommendations. A planned interim analysis was performed upon accrual of 17 cases of invasive disease due to vaccine-type *S. pneumoniae* (August 1998). Ancillary endpoints for evaluation of efficacy against pneumococcal disease were also assessed in this trial.

Efficacy against invasive disease: Invasive disease was defined as isolation and identification of *S. pneumoniae* from normally sterile body sites in children presenting with an acute illness consistent with pneumococcal disease. Weekly surveillance of listings of cultures from the NCKP Regional Microbiology database was conducted to assure ascertainment of all cases. The primary endpoint was efficacy against invasive pneumococcal disease due to vaccine serotypes. The per protocol analysis of the primary endpoint included cases which occurred ≥ 14 days after the third dose. The intent-to-treat (ITT) analysis included all cases of invasive pneumococcal disease due to vaccine serotypes in children who received at least one dose of vaccine. Secondary analyses of efficacy against all invasive pneumococcal disease, regardless of serotype, were also performed according to these same per protocol and ITT definitions. Results of these analyses are presented in Table 1.

TABLE 1
Efficacy of Prevnar™ Against Invasive Disease Due to *S. pneumoniae* in Cases Accrued From October 15, 1995 Through August 20, 1999[*,†]

	Prevnar™ Number of Cases	Control* Number of Cases	Efficacy	95% CI
Vaccine serotypes				
Per protocol	0	17	100%	75.4, 100
Intent-to-treat	0	22	100%	81.7, 100
All pneumococcal serotypes				
Per protocol	2	20	90.0%	58.3, 98.9
Intent-to-treat	3	27†	88.9%	63.8, 97.9

* Investigational meningococcal group C conjugate vaccine (MnCC).
† Includes one case in an immunocompromised subject.

All 22 cases of invasive disease due to vaccine serotype strains in the ITT population were bacteremic. In addition, the following diagnoses were also reported: meningitis (2), pneumonia (2), and cellulitis (1).

Preliminary efficacy data through an extended follow-up period to April 20, 1999, resulted in a similar efficacy estimate (Per protocol: 1 case in Prevnar™ group, 39 cases in control group; ITT: 3 cases in Prevnar™ group, 49 cases in the control group).

Immunogenicity

Routine Schedule

Subjects from a subset of selected study sites in the NCKP efficacy study were approached for participation in the immunogenicity portion of the study on a volunteer basis. Immune responses following three or four doses of Prevnar™ or the control vaccine were evaluated in children who received either concurrent Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed and Haemophilus b Conjugate Vaccine (Diphtheria CRM_{197} Protein Conjugate), (DTP-HbOC), or Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP), and Haemophilus b Conjugate Vaccine (Diphtheria CRM_{197} Protein Conjugate), (HbOC) vaccines at 2, 4, and 6 months of age. The use of Hepatitis B (Hep B), Oral Polio Vaccine (OPV), Inactivated Polio Vaccine (IPV), Measles-Mumps-Rubella (MMR), and Varicella vaccines were permitted according to the AAP and ACIP recommendations.

Table 2 presents the geometric mean concentrations (GMC) of pneumococcal antibodies following the third and fourth doses of Prevnar™ or the control vaccine when administered concurrently with DTP-HbOC vaccine in the efficacy study.

TABLE 2
Geometric Mean Concentrations (µg/mL) of Pneumococcal Antibodies Following the Third and Fourth Doses of Prevnar™ or Control* When Administered Concurrently With DTP-HbOC in the Efficacy Study[¶]

Serotype	Post dose 3 GMC† (95% CI for Prevnar™)		Post dose 4 GMC‡ (95% CI for Prevnar™)	
	Prevnar™§	Control*	Prevnar™§	Control*
	N=88	N=92	N=68	N=61
4	1.46 (1.19, 1.78)	0.03	2.38 (1.88, 3.03)	0.04
6B	4.70 (3.59, 6.14)	0.08	14.45 (11.17, 18.69)	0.17
9V	1.99 (1.64, 2.42)	0.05	3.51 (2.75, 4.48)	0.06
14	4.60 (3.70, 5.74)	0.05	6.52 (5.18, 8.21)	0.06
18C	2.16 (1.73, 2.69)	0.04	3.43 (2.70, 4.37)	0.07
19F	1.39 (1.16, 1.68)	0.09	2.07 (1.66, 2.57)	0.18
23F	1.85 (1.46, 2.34)	0.05	3.82 (2.85, 5.11)	0.09

* Control was investigational meningococcal group C conjugate vaccine (MnCC).
† Mean age of Prevnar™ group was 7.8 months and of control group was 7.7 months. N is slightly less for some serotypes in each group.
‡ Mean age of Prevnar™ group was 14.2 months and of control group was 14.4 months. N is slightly less for some serotypes in each group.
§ p<0.001 when Prevnar™ compared to control for each serotype using a Wilcoxon's test.

In another randomized study (Manufacturing Bridging Study, 118-16), immune responses were evaluated following three doses of Prevnar™ administered concomitantly with DTaP and HbOC vaccines at 2, 4, and 6 months of age, IPV at 2 and 4 months of age, and Hep B at 2 and 6 months of age. The control group received concomitant vaccines only. Table 3 presents the immune responses to pneumococcal polysaccharides observed in both this study and in the subset of subjects from the efficacy study that received concomitant DTaP and HbOC vaccines.

TABLE 3
Geometric Mean Concentrations (µg/mL) of Pneumococcal Antibodies Following the Third Dose of Prevnar™ or Control* When Administered Concurrently With DTaP and HbOC in the Efficacy Study† and Manufacturing Bridging Study[‡,¶]

Serotype	Efficacy Study: Post dose 3 GMC§ (95% CI for Prevnar™)		Manufacturing Bridging Study: Post dose 3 GMC§ (95% CI for Prevnar™)	
	Prevnar™#	Control*	Prevnar™#	Control*
	N=32	N=32	N=159	N=83
4	1.47 (1.08, 2.02)	0.02	2.03 (1.75, 2.37)	0.02
6B	2.18 (1.20, 3.96)	0.06	2.97 (2.43, 3.65)	0.07
9V	1.52 (1.04, 2.22)	0.04	1.18 (1.01, 1.39)	0.04
14	5.05 (3.32, 7.70)	0.04	4.64 (3.80, 5.66)	0.04
18C	2.24 (1.65, 3.02)	0.04	1.96 (1.66, 2.30)	0.04
19F	1.54 (1.09, 2.17)	0.10	1.91 (1.63, 2.25)	0.08
23F	1.48 (0.97, 2.25)	0.05	1.71 (1.44, 2.05)	0.05

* Control in efficacy study was investigational meningococcal group C conjugate vaccine (MnCC) and in Manufacturing Bridging Study was concomitant vaccines only.
† Sufficient data are not available to reliably assess GMCs following 4 doses of Prevnar™ when administrered with DTaP in the NCKP efficacy study.
‡ Mean age of the Prevnar™ group was 7.4 months and of the control group was 7.6 months. N is slightly less for some serotypes in each group.
§ Mean age of the Prevnar™ group and the control group was 7.2 months.
¶ p<0.001 when Prevnar™ compared to control for each serotype using a Wilcoxon's test in the efficacy study and two-sample t-test in the Manufacturing Bridging Study.

In all studies in which the immune responses to Prevnar™ were contrasted to control, a significant antibody response was seen to all vaccine serotypes following three or four doses, although geometric mean concentrations of antibody varied among serotypes.[1,2,3,4,5,6,7,8,9,10,11,12] The minimum serum antibody concentration necessary for protection against invasive pneumococcal disease has not been determined for any serotype.

Prevnar™ induces functional antibodies to all vaccine serotypes, as measured by opsonophagocytosis following three doses.[13]

Previously Unvaccinated Older Infants and Children

To determine an appropriate schedule for children 7 months of age or older at the time of the first immunization with Prevnar™, 483 children in 4 ancillary studies received Prevnar™ at various schedules. GMCs attained using the various schedules among older infants and children were comparable to immune responses of children, who received concomitant DTaP, in the NCKP efficacy study (118-8) after 3 doses for most serotypes, as shown in Table 4. These data support the schedule for previously unvaccinated older infants and children who are beyond the age of the infant schedule. For usage in older infants and children see DOSAGE AND ADMINISTRATION.

TABLE 4
Geometric Mean Concentrations (µg/mL) of Pneumococcal Antibodies Following Immunization of Children From 7 Months Through 9 Years of Age With Prevnar™ [13]

Age group, Vaccinations	Study	Sample Size(s)	4	6B	9V	14	18C	19F	23F
7-11 mo. 3 doses	118-12	22	2.34	3.66	2.11	9.33	2.31	1.60	2.50
	118-16	39	3.60	4.63	2.04	5.48	1.98	2.15	1.93
12-17 mo. 2 doses	118-15*	82-84†	3.91	4.67	1.94	6.92	2.25	3.78	3.29
	118-18	33	7.02	4.25	3.26	6.31	3.60	3.29	2.92
18-23 mo. 2 doses	118-15*	52-54†	3.36	4.92	1.80	6.69	2.65	3.17	2.71
	118-18	45	6.85	3.71	3.86	6.48	3.42	3.86	2.75
24-35 mo. 1 dose	118-18	53	5.34	2.90	3.43	1.88	3.03	4.07	1.56
36-59 mo. 1 dose	118-18	52	6.27	6.40	4.62	5.95	4.08	6.37	2.95
5-9 yrs. 1 dose	118-18	101	6.92	20.84	7.49	19.32	6.72	12.51	11.57
118-8, DTaP	Post dose 3	31-32†	1.47	2.18	1.52	5.05	2.24	1.54	1.48

Bold = GMC not inferior to 118-8, DTaP post dose 3 (one-sided lower limit of the 95% CI of GMC ratio ≥ 0.50).

* Study in Navajo and Apache populations.

† Numbers vary with serotype.

INDICATIONS AND USAGE

Prevnar™ is indicated for active immunization of infants and toddlers against invasive disease caused by *S. pneumoniae* due to capsular serotypes included in the vaccine (4, 6B, 9V, 14, 18C, 19F, and 23F). The routine schedule is 2, 4, 6, and 12-15 months of age. For additional information on usage, see DOSAGE AND ADMINISTRATION.

This vaccine is not intended to be used for treatment of active infection.

As with any vaccine, Prevnar™ may not protect 100% of individuals receiving the vaccine.

CONTRAINDICATIONS

Hypersensitivity to any component of the vaccine, including diphtheria toxoid, is a contraindication to use of this vaccine.

The decision to administer or delay vaccination because of a current or recent febrile illness depends largely on the severity of the symptoms and their etiology. Although a severe or even a moderate febrile illness is sufficient reason to postpone vaccinations, minor illnesses, such as a mild upper respiratory infection with or without low-grade fever, are not generally contraindications.[27,28]

WARNINGS

THIS VACCINE WILL NOT PROTECT AGAINST *S. PNEUMONIAE* DISEASE OTHER THAN THAT CAUSED BY THE SEVEN SEROTYPES INCLUDED IN THE VACCINE, NOR WILL IT PROTECT AGAINST OTHER MICROORGANISMS THAT CAUSE INVASIVE INFECTION SUCH AS BACTEREMIA AND MENINGITIS.

This vaccine should not be given to infants or children with thrombocytopenia or any coagulation disorder that would contraindicate intramuscular injection unless the potential benefit clearly outweighs the risk of administration. If the decision is made to administer this vaccine to children with coagulation disorders, it should be given with caution. (See DRUG INTERACTIONS).

Immunization with Prevnar™ does not substitute for routine diphtheria immunization.

Healthcare professionals should prescribe and/or administer this product with caution to patients with a possible history of latex sensitivity since the packaging contains dry natural rubber.

PRECAUTIONS

Prevnar™ is for intramuscular use only. Prevnar™ SHOULD UNDER NO CIRCUMSTANCES BE ADMINISTERED INTRAVENOUSLY. The safety and immunogenicity for other routes of administration (e.g. subcutaneous) have not been evaluated.

General

CARE IS TO BE TAKEN BY THE HEALTHCARE PROFESSIONAL FOR THE SAFE AND EFFECTIVE USE OF THIS PRODUCT.

1. PRIOR TO ADMINISTRATION OF ANY DOSE OF THIS VACCINE, THE PARENT OR GUARDIAN SHOULD BE ASKED ABOUT THE PERSONAL HISTORY, FAMILY HISTORY, AND RECENT HEALTH STATUS OF THE VACCINE RECIPIENT. THE HEALTHCARE PROFESSIONAL SHOULD ASCERTAIN PREVIOUS IMMUNIZATION HISTORY, CURRENT HEALTH STATUS, AND OCCURRENCE OF ANY SYMPTOMS AND/OR SIGNS OF AN ADVERSE EVENT AFTER PREVIOUS IMMUNIZATIONS IN THE CHILD TO BE IMMUNIZED, IN ORDER TO DETERMINE THE EXISTENCE OF ANY CONTRAINDICATION TO IMMUNIZATION WITH THIS VACCINE AND TO ALLOW AN ASSESSMENT OF RISKS AND BENEFITS.
2. BEFORE THE ADMINISTRATION OF ANY BIOLOGICAL, THE HEALTHCARE PROFESSIONAL SHOULD TAKE ALL PRECAUTIONS KNOWN FOR THE PREVENTION OF ALLERGIC OR ANY OTHER ADVERSE REACTIONS. This should include a review of the patient's history regarding possible sensitivity; the ready availability of epinephrine 1:1000 and other appropriate agents used for control of immediate allergic reactions; and a knowledge of the recent literature pertaining to use of the biological concerned, including the nature of side effects and adverse reactions that may follow its use.
3. Children with impaired immune responsiveness, whether due to the use of immunosuppressive therapy (including irradiation, corticosteroids, antimetabolites, alkylating agents, and cytotoxic agents), a genetic defect, HIV infection, or other causes, may have reduced antibody response to active immunization.[27,29] (See DRUG INTERACTIONS).
4. The use of pneumococcal conjugate vaccine does not replace the use of 23-valent pneumococcal polysaccharide vaccine in children ≥ 24 months of age with sickle cell disease, asplenia, HIV infection, chronic illness or who are immunocompromised. Data on sequential vaccination with Prevnar™ followed by 23-valent pneumococcal polysaccharide vaccine are limited. In a randomized study, 23 children ≥ 2 years of age with sickle cell disease were administered either 2 doses of Prevnar™ followed by a dose of polysaccharide vaccine or a single dose of polysaccharide vaccine alone. In this small study, safety and immune responses with the combined schedule were similar to polysaccharide vaccine alone.[30]
5. Since this product is a suspension containing an aluminum adjuvant, shake vigorously immediately prior to use to obtain a uniform suspension prior to withdrawing the dose.
6. A separate sterile syringe and needle or a sterile disposable unit should be used for each individual to prevent transmission of hepatitis or other infectious agents from one person to another. Needles should be disposed of properly and should not be recapped.
7. Special care should be taken to prevent injection into or near a blood vessel or nerve.
8. Healthcare professionals should prescribe and/or administer this product with caution to patients with a possible history of latex sensitivity since the packaging contains dry natural rubber.

Information for Parents or Guardians

Prior to administration of this vaccine, the healthcare professional should inform the parent, guardian, or other responsible adult of the potential benefits and risks to the patient (see ADVERSE REACTIONS and WARNINGS sections), and the importance of completing the immunization series unless contraindicated. Parents or guardians should be instructed to report any suspected adverse reactions to their healthcare professional. The healthcare professional should provide vaccine information statements prior to each vaccination.

DRUG INTERACTIONS

Children receiving therapy with immunosuppressive agents (large amounts of corticosteroids, antimetabolites, alkylating agents, cytotoxic agents) may not respond optimally to active immunization.[27,29,31] (See PRECAUTIONS, General).

As with other intramuscular injections, Prevnar™ should be given with caution to children on anticoagulant therapy.

Simultaneous Administration with Other Vaccines

During clinical studies, Prevnar™ was administered simultaneously with DTP-HbOC or DTaP and HbOC, OPV or IPV, Hep B vaccines, MMR, and Varicella vaccine. Thus, the safety experience with Prevnar™ reflects the use of this product as part of the routine immunization schedule.[1,2,3,4,5,6,7,8,9,10,11,12]

The immune response to routine vaccines when administered with Prevnar™ (at separate sites) was assessed in 3 clinical studies in which there was a control group for comparison. Results for the concurrent immunizations in infants are shown in Table 5 and for toddlers in Table 6. Enhancement of antibody response to HbOC in the infant series was observed. Some suppression of *Haemophilus influenzae* type b (Hib) response was seen at the 4th dose, but over 97% of children achieved titers ≥ 1 µg/mL. Although some inconsistent differences in response to pertussis antigens were observed, the clinical relevance is unknown. The response to 2 doses of IPV given concomitantly with Prevnar™, assessed 3 months after the second dose, was equivalent to controls for poliovirus Types 2 and 3, but lower for Type 1. MMR and Varicella immunogenicity data from controlled clinical trials with concurrent administration of Prevnar™ are not available.

TABLE 5
Concurrent Administration of Prevnar™ With Other Vaccines to Infants in Non-Efficacy Studies[14,15]

Antigen*	GMC*		% Responders†		Study	Vaccine Schedule‡	N	
	Prevnar™	Control§	Prevnar™	Control§		(mo.)	Prevnar™	Control§
Hib	6.2	4.4	99.5, 88.3	97.0, 88.1	118-12	2,4,6	214	67
Diphtheria	0.9	0.8	100	97.0				
Tetanus	3.5	4.1¶	100	100				
PT	19.1	17.8	74.0	69.7				
FHA	43.8	46.7	66.4	69.7				
Pertactin	40.1	50.9	65.6	77.3				
Fimbriae 2	3.3	4.2	44.7	62.5¶				
Hib	11.9	7.8¶	100, 96.9	98.8, 92.8	118-16	2,4,6	159	83
Hep B	–	–	99.4	96.2	118-16	0,2,6	156	80
IPV Type 1	–	–	89.0	93.6¶	118-16	2,4	156	80
Type 2	–	–	94.2	93.6				
Type 3	–	–	83.8	80.8				

* Hib vaccine was HibTITER®, DTaP vaccine was Acel-Imune®. Hib (µg/mL); Dip, Tet (IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) (units/mL).

† Responders = Hib (≥0.15 µg/mL, ≥1.0 µg/mL); Dip, Tet (≥0.1 IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) [4-fold rise]; IPV (≥1:10); Hep B (≥10 mIU/mL).

‡ Schedule for concurrently administered vaccines; Prevnar™ administered at 2, 4, 6 mos.; blood for antibody assessment attained 1 month after third dose, except for IPV (3 months post-immunization).

§ Concurrent vaccines only.

¶ $p<0.05$ when Prevnar™ compared to control group using the following tests: ANCOVA for GMCs in 118-12; ANOVA for GMCs in 118-16; and Fisher's Exact test for % Responders in 118-12.

Lower bound of 90% CI of difference >10%.

TABLE 6
Concurrent Administration of Prevnar™ With Other Vaccines to Toddlers in a Non-Efficacy Study[16]

Antigen*	GMC*		% Responders†		Study‡	Vaccine Schedule§	N	
	Prevnar™	Control¶	Prevnar™	Control¶		(mo.)	Prevnar™	Control¶
Hib	22.7	47.9#	100, 97.9	100, 100	118-7	12-15	47	26
Diphtheria	2.0	3.2#	100	100				
Tetanus	14.4	18.8	100	100				
PT	68.6	121.2#	68.1	73.1				
FHA	29.0	48.2#	68.1	84.6				
Pertactin	84.4	83.0	83.0	96.2				
Fimbriae 2	5.2	3.8	63.8	50.0				

* Hib vaccine was HibTITER®, DTaP vaccine was Acel-Imune®. Hib (µg/mL); Dip, Tet (IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) (units/mL).

† Responders = Hib (≥0.15 µg/mL, ≥1.0 µg/mL); Dip, Tet (≥0.1 IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) [4-fold rise].

‡ Children received a primary series of DTP-HbOC (Tetramune®).

§ Blood for antibody assessment obtained 1 month after dose.

¶ Concurrent vaccines only.

# $p<0.05$ when Prevnar™ compared to control group using a two-sample t-test.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Prevnar™ has not been evaluated for any carcinogenic or mutagenic potential, or impairment of fertility.

PREGNANCY

Pregnancy Category C

Animal reproductive studies have not been conducted with this product. It is not known whether Prevnar™ can cause fetal harm when administered to a pregnant woman or whether it can affect reproductive capacity. This vaccine is not recommended for use in pregnant women.

Nursing Mothers

It is not known whether vaccine antigens or antibodies are excreted in human milk. This vaccine is not recommended for use in a nursing mother.

PEDIATRIC USE

Prevnar™ has been shown to be usually well-tolerated and immunogenic in infants. The safety and effectiveness of Prevnar™ in children below the age of 6 weeks have not been established. Immune responses elicited by Prevnar™ among infants born prematurely have not been studied. See DOSAGE AND ADMINISTRATION for the recommended pediatric dosage.

GERIATRIC USE

This vaccine is NOT recommended for use in adult populations. It is not to be used as a substitute for the pneumococcal polysaccharide vaccine, in geriatric populations.

ADVERSE REACTIONS

The majority of the safety experience with Prevnar™ comes from the NCKP Efficacy Trial in which 17,066 infants received 55,352 doses of Prevnar™, along with other routine childhood vaccines through April 19 (see CLINICAL PHARMACOLOGY section). The number of Prevnar™ recipients in the safety analysis diff

from the number included in the efficacy analysis due to the different lengths of follow-up for these study endpoints. Safety was monitored in this study using several modalities. Local reactions and systemic events occurring within 48 hours of each dose of vaccine were ascertained by scripted telephone interview on a randomly selected subset of approximately 3,000 children in each vaccine group. The rate of relatively rare events requiring medical attention was evaluated across all doses in all study participants using automated databases. Specifically, rates of hospitalizations within 3, 14, 30, and 60 days of immunization, and of emergency room visits within 3, 14, and 30 days of immunization were assessed and compared between vaccine groups for each diagnosis. Seizures within 3 and 30 days of immunization were ascertained across multiple settings (hospitalizations, emergency room or clinic visits, telephone interviews). Deaths and SIDS were ascertained through April 1999. Hospitalizations due to diabetes, autoimmune disorders, and blood disorders were ascertained through August 1999.

In Tables 7 and 8, the rate of local reactions at the Prevnar™ injection site is compared at each dose to the DTP or DTaP injection site in the same children.

TABLE 7
Percentage of Subjects Reporting Local Reactions Within 2 Days Following Immunization With Prevnar™ and DTP-HbOC* Vaccines at 2, 4, 6, and 12-15 Months of Age

Reaction	Dose 1		Dose 2		Dose 3		Dose 4	
	Prevnar™ Site	DTP-HbOC Site†	Prevnar™ Site	DTP-HbOC Site†	Prevnar™ Site	DTP-HbOC Site†	Prevnar™ Site	DTP-HbOC Site†
	N=2890	N=2890	N=2725	N=2725	N=2538	N=2538	N=599	N=599
Erythema								
Any	12.4	21.9	14.3	25.1	15.2	26.5	12.7	23.4
> 2.4 cm	1.2	4.6	1.0	2.9	2.0	4.4	1.7	6.4
Induration								
Any	10.9	22.4	12.3	23.0	12.8	23.3	11.4	20.5
> 2.4 cm	2.6	7.2	2.4	5.6	2.9	6.7	2.8	7.2
Tenderness								
Any	28.0	36.4	25.2	30.5	25.6	32.8	36.5	45.1
Interfered with limb movement	7.9	10.7	7.4	8.4	7.8	10.0	18.5	22.2

* If Hep B vaccine was administered simultaneously, it was administered into the same limb as the DTP-HbOC vaccine. If reactions occurred at either or both sites on that limb, the more severe reaction was recorded.
† $p<0.05$ when Prevnar™ site compared to the DTP-HbOC site using the sign test.

TABLE 8
Percentage of Subjects Reporting Local Reactions Within 2 Days Following Immunization With Prevnar™* and DTaP Vaccines† at 2, 4, 6, and 12-15 Months of Age

Reaction	Dose 1		Dose 2		Dose 3		Dose 4	
	Prevnar™ Site	DTaP Site	Prevnar™ Site	DTaP Site	Prevnar™ Site	DTaP Site	Prevnar™ Site	DTaP Site‡
	N=693	N=693	N=526	N=526	N=422	N=422	N=165	N=165
Erythema								
Any	10.0	6.7§	11.6	10.5	13.8	11.4	10.9	3.6§
> 2.4 cm	1.3	0.4§	0.6	0.6	1.4	1.0	3.6	0.6
Induration								
Any	9.8	6.6§	12.0	10.5	10.4	10.4	12.1	5.5§
> 2.4 cm	1.6	0.9	1.3	1.7	2.4	1.9	5.5	1.8
Tenderness								
Any	17.9	16.0	19.4	17.3	14.7	13.1	23.3	18.4
Interfered with limb movement	3.1	1.8§	4.1	3.3	2.9	1.9	9.2	8.0

* HbOC was administered in the same limb as Prevnar™. If reactions occurred at either or both sites on that limb, the more severe reaction was recorded.
† If Hep B vaccine was administered simultaneously, it was administered into the same limb as DTaP. If reactions occurred at either or both sites on that limb, the more severe reaction was recorded.
‡ Subjects may have received DTP or a mixed DTP/DTaP regimen for the primary series. Thus, this is the 4th dose of a pertussis vaccine, but not a 4th dose of DTaP.
§ $p<0.05$ when Prevnar™ site compared to DTaP site using the sign test.

Table 9 presents the rates of local reactions in previously unvaccinated older infants and children.

TABLE 9
Percentage of Subjects Reporting Local Reactions Within 3 Days of Immunization in Infants and Children from 7 Months Through 9 Years of Age

Age at 1st Vaccination	7 - 11 Mos.						12 - 23 Mos.			24 - 35 Mos.	36 - 59 Mos.	5 - 9 Yrs.
Study No.	118-12			118-16			118-9*	118-18		118-18	118-18	118-18
Dose Number	1	2	3†	1	2	3†	1	1	2	1	1	1
Number of Subjects	54	51	24	81	76	50	60	114	117	46	48	49
Reaction												
Erythema												
Any	16.7	11.8	20.8	7.4	7.9	14.0	48.3	10.5	9.4	6.5	29.2	24.2
> 2.4 cm‡	1.9	0.0	0.0	0.0	0.0	0.0	6.7	1.8	1.7	0.0	8.3	7.1
Induration												
Any	16.7	11.8	8.3	7.4	3.9	10.0	48.3	8.8	6.0	10.9	22.9	25.5
> 2.4 cm‡	3.7	0.0	0.0	0.0	0.0	0.0	3.3	0.9	0.9	2.2	6.3	9.3
Tenderness												
Any	13.0	11.8	12.5	8.6	10.5	12.0	46.7	25.7	26.5	41.3	58.3	82.8
Interfered with limb movement§	1.9	2.0	4.2	1.2	1.3	0.0	3.3	6.2	8.5	13.0	20.8	39.4

* For 118-9, 2 of 60 subjects were ≥24 months of age.
† For 118-12, dose 3 was administered at 15 - 18 mos. of age. For 118-16, dose 3 was administered at 12 - 15 mos. of age.
‡ For 118-16 and 118-18, ≥2 cm.
§ Tenderness interfering with limb movement.

Tables 10 and 11 present the rates of systemic events observed in the efficacy study when Prevnar™ was administered concomitantly with DTP or DTaP.

TABLE 10
Percentage of Subjects* Reporting Systemic Events Within 2 Days Following Immunization With Prevnar™ or Control† Vaccine Concurrently With DTP-HbOC Vaccine at 2, 4, 6, and 12-15 Months of Age[15]

Reaction	Dose 1		Dose 2		Dose 3		Dose 4	
	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†
	N=2998	N=2982	N=2788	N=2761	N=2596	N=2591	N=709	N=733
Fever								
≥ 38.0°C	33.4	28.7‡	34.7	27.4‡	40.6	32.4‡	41.9	36.9
> 39.0°C	1.3	1.3	3.0	1.6‡	5.3	3.4‡	4.5	4.5
Irritability	71.3	67.9‡	69.4	63.8‡	68.9	61.6‡	72.8	65.8‡
Drowsiness	49.2	50.6	32.5	33.6	25.9	23.4‡	21.3	22.7
Restless Sleep	18.1	17.9	27.3	24.3‡	33.3	30.1‡	29.9	28.0
Decreased Appetite	24.7	23.6	22.8	20.3‡	27.7	25.6	33.0	27.4‡
Vomiting	17.9	14.9‡	16.2	14.4	15.5	12.7‡	9.6	6.8
Diarrhea	12.0	10.7	10.9	9.9	11.5	10.4	12.1	11.2
Rash or Hives	0.7	0.6	0.8	0.8	1.4	1.1	1.4	0.8

* Approximately 90% of subjects received prophylactic or therapeutic antipyretics within 48 hours of each dose.
† Investigational meningococcal group C conjugate vaccine (MnCC).
‡ p<0.05 when Prevnar™ compared to control group using a Chi-Square test.

TABLE 11
Percentage of Subjects* Reporting Systemic Events Within 2 Days Following Immunization With Prevnar™ or Control† Vaccine Concurrently With DTaP Vaccine at 2, 4, 6, and 12-15 Months of Age[15]

Reaction	Dose 1		Dose 2		Dose 3		Dose 4‡	
	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†
	N=710	N=711	N=559	N=508	N=461	N=414	N=224	N=230
Fever								
≥ 38.0°C	15.1	9.4§	23.9	10.8§	19.1	11.8§	21.0	17.0
> 39.0°C	0.9	0.3	2.5	0.8§	1.7	0.7	1.3	1.7
Irritability	48.0	48.2	58.7	45.3§	51.2	44.8	44.2	42.6
Drowsiness	40.7	42.0	25.6	22.8	19.5	21.9	17.0	16.5
Restless Sleep	15.3	15.1	20.2	19.3	25.2	19.0§	20.2	19.1
Decreased Appetite	17.0	13.5	17.4	13.4	20.7	13.8§	20.5	23.1
Vomiting	14.6	14.5	16.8	14.4	10.4	11.6	4.9	4.8
Diarrhea	11.9	8.4§	10.2	9.3	8.3	9.4	11.6	9.2
Rash or Hives	1.4	0.3§	1.3	1.4	0.4	0.5	0.5	1.7

* Approximately 75% of subjects received prophylactic or therapeutic antipyretics within 48 hours of each dose.
† Investigational meningococcal group C conjugate vaccine (MnCC).
‡ Most of these children had received DTP for the primary series. Thus, this is a 4th dose of a pertussis vaccine, but not of DTaP.
§ p<0.05 when Prevnar™ compared to control group using a Chi-Square test.

Table 12 presents results from a second study (Manufacturing Bridging Study) conducted at Northern California and Denver Kaiser sites, in which children were randomized to receive one of three lots of Prevnar™ with concomitant vaccines including DTaP, or the same concomitant vaccines alone. Information was ascertained by scripted telephone interview, as described above.

TABLE 12
Percentage of Subjects* Reporting Systemic Reactions Within 3 Days Following Immunization With Prevnar™, DTaP, HbOC, Hep B, and IPV vs. Control† in Manufacturing Bridging Study[15]

Reaction	Dose 1		Dose 2		Dose 3	
	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†
	N=498	N=108	N=452	N=99	N=445	N=89
Fever						
≥ 38.0°C	21.9	10.2‡	33.6	17.2‡	28.1	23.6
> 39.0°C	0.8	0.9	3.8	0.0	2.2	0.0
Irritability	59.7	60.2	65.3	52.5‡	54.2	50.6
Drowsiness	50.8	38.9‡	30.3	31.3	21.2	20.2
Decreased Appetite	19.1	15.7	20.6	11.1‡	20.4	9.0‡

* Approximately 72% of subjects received prophylactic or therapeutic antipyrectics within 48 hours of each dose.
† Control group received concomitant vaccines only in the same schedule as the Prevnar™ group (DTaP, HbOC at dose 1, 2, 3; IPV at doses 1 and 2; Hep B at doses 1 and 3).
‡ p<0.05 when Prevnar™ compared to control group using Fisher's Exact test.

Fever (≥ 38.0°C) within 48 hours of a vaccine dose was reported by a greater proportion of subjects who received Prevnar™, compared to control (investigational meningococcal group C conjugate vaccine [MnCC]), after each dose when administered concurrently with DTP-HbOC or DTaP in the efficacy study. In the Manufacturing Bridging Study, fever within 48-72 hours was also reported more commonly after each dose compared to infants in the control group who received only recommended vaccines. When administered concurrently with DTaP in either study, fever rates among Prevnar™ recipients ranged from 15% to 34%, and were greatest after the 2nd dose.

Table 13 presents the frequencies of systemic reactions in previously unvaccinated older infants and childre

TABLE 13
Percentage of Subjects Reporting Systemic Reactions Within 3 Days of Immunization in Infants and Children from 7 Months Through 9 Years of Age[15]

Age at 1st Vaccination	7 - 11 Mos.						12 - 23 Mos.			24 - 35 Mos.	36 - 59 Mos.	5 - Yrs
Study No.	118-12			118-16			118-9*	118-18		118-18	118-18	118-
Dose Number	1	2	3†	1	2	3†	1	1	2	1	1	1
Number of Subjects	54	51	24	85	80	50	60	120	117	47	52	10
Reaction												
Fever												
≥ 38.0°C	20.8	21.6	25.0	17.6	18.8	22.0	36.7	11.7	6.8	14.9	11.5	7.(
> 39.0°C	1.9	5.9	0.0	1.6	3.9	2.6	0.0	4.4	0.0	4.2	2.3	1.2
Fussiness	29.6	39.2	16.7	54.1	41.3	38.0	40.0	37.5	36.8	46.8	34.6	29.3
Drowsiness	11.1	17.6	16.7	24.7	16.3	14.0	13.3	18.3	11.1	12.8	17.3	11.(
Decreased Appetite	9.3	15.7	0.0	15.3	15.0	30.0	25.0	20.8	16.2	23.4	11.5	9.(

* For 118-9, 2 of 60 subjects were ≥24 months of age.
† For 118-12, dose 3 was administered at 15 - 18 mos. of age. For 118-16, dose 3 was administered a 12 - 15 mos. of age.

Of the 17,066 subjects who received at least one dose of Prevnar™ in the efficacy trial, there were 24 hc pitalizations (for 29 diagnoses) within 3 days of a dose from October 1995 through April 1998. Diagnos were as follows: bronchiolitis (5); congenital anomaly (4); elective procedure, UTI (3 each); acute gastroenteritis, asthma, pneumonia (2 each); aspiration, breath holding, influenza, inguinal hernia repair, ot media, febrile seizure, viral syndrome, well child/reassurance (1 each). There were 162 visits to the eme gency room (for 182 diagnoses) within 3 days of a dose from October 1995 through April 1998. Diagnoses were as follows: febrile illness (20); acute gastroenteritis (19); trauma, URI (16 each); otitis media (15); well child (13); irritable child, viral syndrome (10 each); rash (8); croup, pneumonia (6 each poisoning/ingestion (5); asthma, bronchiolitis (4 each); febrile seizure, UTI (3 each); thrush, wheezing, breath holding, choking, conjunctivitis, inguinal hernia repair, pharyngitis (2 each); colic, colitis, congest heart failure, elective procedure, hives, influenza, ingrown toenail, local swelling, roseola, sepsis (1 each

One case of a hypotonic-hyporesponsive episode (HHE) was reported in the efficacy study following Prevnar™ and concurrent DTP vaccines in the study period from October 1995 through April 1998. Two additional cases of HHE were reported in four other studies and these also occurred in children who received Prevnar™ concurrently with DTP vaccine.[22,23]

In the Kaiser efficacy study in which 17,066 children received a total of 55,352 doses of Prevnar™ and 17,080 children received a total of 55,387 doses of the control vaccine (investigational meningococcal group C conjugate vaccine [MnCC]), seizures were reported in 8 Prevnar™ recipients and 4 control vacc recipients within 3 days of immunization from October 1995 through April 1998. Of the 8 Prevnar™ rec ents, 7 received concomitant DTP-containing vaccines and one received DTaP. Of the 4 control vaccine recipients, 3 received concomitant DTP-containing vaccines and one received DTaP.[15] In the other 4 stuc combined, in which 1,102 children were immunized with 3,347 doses of Prevnar™ and 408 children we immunized with 1,310 doses of control vaccine (either investigational meningococcal group C conjugat vaccine [MnCC] or concurrent vaccines), there was one seizure event reported within 3 days of immuni tion.[23] This subject received Prevnar™ concurrent with DTaP vaccine.

Twelve deaths (5 SIDS and 7 with clear alternative cause) occurred among subjects receiving Prevnar™ which 11 (4 SIDS and 7 with clear alternative cause) occurred in the Kaiser efficacy study from October 1995 until April 20, 1999. In comparison, 21 deaths (8 SIDS, 12 with clear alternative cause and one SIDS-like death in an older child), occurred in the control vaccine group during the same time period in efficacy study.[15,23] The number of SIDS deaths in the efficacy study from October 1995 until April 20, 19 was similar to or lower than the age and season-adjusted expected rate from the California State data fr 1995-1997 and are presented in Table 14.

TABLE 14
Age and Season Adjusted Comparison of SIDS Rates in the NCKP Efficacy Trial With the Expected Rate from the California State Data for 1995-1997[15]

Vaccine	< One Week After Immunization		≤ Two Weeks After Immunization		≤ One Month After Immunization		≤ One Year Afte Immunization	
	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs
Prevnar™	1.06	1	2.09	2	4.28	2	8.08	4
Control*	1.06	2	2.09	3†	4.28	3†	8.08	8†

* Investigational meningococcal group C conjugate vaccine (MnCC).
† Does not include one additional case of SIDS-like death in a child older than the usual SIDS age (448 days).

In a review of all hospitalizations that occurred between October 1995 and August 1999 in the efficacy st for the specific diagnoses of aplastic anemia, autoimmune disease, autoimmune hemolytic anemia, diabe mellitus, neutropenia, and thrombocytopenia, the numbers of such cases were either equal to or less tha the expected numbers based on the 1995 Kaiser Vaccine Safety Data Link (VSD) data set.

Overall, the safety of Prevnar™ was evaluated in a total of five clinical studies in which 18,168 infants anc children received a total of 58,699 doses of vaccine at 2, 4, 6, and 12-15 months of age. In addition, the safety of Prevnar™ was evaluated in 560 children from 4 ancillary studies who started immunization at 7 months to 9 years of age. Tables 15 and 16 summarize systemic reactogenicity data within 2 or 3 days across 4,748 subjects (13,039 infant doses and 1,706 toddler doses) for whom these data were collecte and according to the pertussis vaccine administered concurrently.

TABLE 15
Overall Percentage of Doses Associated With Systemic Events Within 2 or 3 Days For Efficacy Study and All Ancillary Studies When Prevnar™ Administered To Infants As a Primary Series at 2, 4, and 6 Months of Age[5,8,20,21,23]

Systemic Event	Prevnar™ Concurrently With DTP-HbOC (9,191 Doses)*	Prevnar™ Concurrently With DTaP and HbOC (3,848 Doses)†	DTaP and HbOC Control (538 Doses)‡
Fever			
≥ 38.0°C	35.6	21.1	14.2
> 39.0°C	3.1	1.8	0.4
Irritability	69.1	52.5	45.2
Drowsiness	36.9	32.9	27.7
Restless Sleep	25.8	20.6	22.3
Decreased Appetite	24.7	18.1	13.6
Vomiting	16.2	13.4	9.8
Diarrhea	11.4	9.8	4.4
Rash or Hives	0.9	0.6	0.3

* Total from which reaction data are available varies between reactions from 8,874-9,191 doses. Data from studies 118-3, 118-7, 118-8.
† Total from which reaction data are available varies between reactions from 3,121-3,848 doses. Data from studies 118-8, 118-12, 118-16.
‡ Total from which reaction data are available varies between reactions from 295-538 doses. Data from studies 118-12 and 118-16.

TABLE 16
Overall Percentage of Doses Associated With Systemic Events Within 2 or 3 Days For Efficacy Study and All Ancillary Studies When Prevnar™ Administered To Toddlers as a Fourth Dose At 12 to 15 Months of Age[5,22]

Systemic Event	Prevnar™ Concurrently With DTP-HbOC (709 Doses)*	Prevnar™ Concurrently With DTaP and HbOC (270 Doses)†	Prevnar™ Only No Concurrent Vaccines (727 Doses)‡
Fever			
≥ 38.0°C	41.9	19.6	13.4
> 39.0°C	4.5	1.5	1.2
Irritability	72.8	45.9	45.8
Drowsiness	21.3	17.5	15.9
Restless Sleep	29.9	21.2	21.2
Decreased Appetite	33.0	21.1	18.3
Vomiting	9.6	5.6	6.3
Diarrhea	12.1	13.7	12.8
Rash or Hives	1.4	0.7	1.2

* Total from which reaction data are available varies between reactions from 706-709 doses. Data from study 118-8.
† Total from which reaction data are available varies between reactions from 269-270 doses. Data from studies 118-7 and 118-8.
‡ Total from which reaction data are available varies between reactions from 725-727 doses. Data from studies 118-7 and 118-8.

With vaccines in general, including Prevnar™, it is not uncommon for patients to note within 48 to 72 hours at or around the injection site the following minor reactions: edema; pain or tenderness; redness, inflammation or skin discoloration; mass; or local hypersensitivity reaction. Such local reactions are usually self-limited and require no therapy.

As with other aluminum-containing vaccines, a nodule may occasionally be palpable at the injection site for several weeks.[20]

ADVERSE EVENT REPORTING

Any suspected adverse events following immunization should be reported by the healthcare professional to the US Department of Health and Human Services (DHHS). The National Vaccine Injury Compensation Program requires that the manufacturer and lot number of the vaccine administered be recorded by the healthcare professional in the vaccine recipient's permanent medical record (or in a permanent office log or file), along with the date of administration of the vaccine and the name, address, and title of the person administering the vaccine.

The US DHHS has established the Vaccine Adverse Event Reporting System (VAERS) to accept all reports of suspected adverse events after the administration of any vaccine including, but not limited to, the reporting of events required by the National Childhood Vaccine Injury Act of 1986. The FDA web site is: http://www.fda.gov/cber/vaers/vaers.htm.

The VAERS toll-free number for VAERS forms and information is 800-822-7967.[24]

DOSAGE AND ADMINISTRATION

For intramuscular injection only. *Do not inject intravenously.*

The dose is 0.5 mL to be given intramuscularly.

Since this product is a suspension containing an adjuvant, shake vigorously immediately prior to use to obtain a uniform suspension in the vaccine container. The vaccine should not be used if it cannot be resuspended.

After shaking, the vaccine is a homogeneous, white suspension.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration (see DESCRIPTION). This product should not be used if particulate matter or discoloration is found.

The vaccine should be injected intramuscularly. The preferred sites are the anterolateral aspect of the thigh in infants or the deltoid muscle of the upper arm in toddlers and young children. The vaccine should not be injected in the gluteal area or areas where there may be a major nerve trunk and/or blood vessel. Before injection, the skin at the injection site should be cleansed and prepared with a suitable germicide. After insertion of the needle, aspirate and wait to see if any blood appears in the syringe, which will help avoid inadvertent injection into a blood vessel. If blood appears, withdraw the needle and prepare for a new injection at another site.

Vaccine Schedule

For infants, the immunization series of Prevnar™ consists of three doses of 0.5 mL each, at approximately 2-month intervals, followed by a fourth dose of 0.5 mL at 12-15 months of age. The customary age for the first dose is 2 months of age, but it can be given as young as 6 weeks of age. The recommended dosing interval is 4 to 8 weeks. The fourth dose should be administered at least 2 months after the third dose.

Previously Unvaccinated Older Infants and Children

For previously unvaccinated older infants and children, who are beyond the age of the routine infant schedule, the following schedule applies:[20]

Age at First Dose	Total Number of 0.5 mL Doses
7-11 months of age	3*
12-23 months of age	2†
≥ 24 months through 9 years of age	1

* 2 doses at least 4 weeks apart; third dose after the one-year birthday, separated from the second dose by at least 2 months.
† 2 doses at least 2 months apart.

(See CLINICAL PHARMACOLOGY section for the limited available immunogenicity data and ADVERSE EVENTS section for limited safety data corresponding to the previously noted vaccination schedule for older children).

Safety and immunogenicity data are either limited or not available for children in specific high risk groups for invasive pneumococcal disease (e.g. persons with sickle cell disease, asplenia, HIV-infected).

HOW SUPPLIED

Vial, 1 Dose (5 per package) - NDC 0005-1970-67

CPT Code 90669

STORAGE

DO NOT FREEZE. STORE REFRIGERATED, AWAY FROM FREEZER COMPARTMENT, AT 2°C TO 8°C (36°F TO 46°F).

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Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein)

Prevnar™

℞ only

For Intramuscular Injection Only

DESCRIPTION

Prevnar™, Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein), is a sterile solution of saccharides of the capsular antigens of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F individually conjugated to diphtheria CRM_{197} protein. Each serotype is grown in soy peptone broth. The individual polysaccharides are purified through centrifugation, precipitation, ultrafiltration, and column chromatography. The polysaccharides are chemically activated to make saccharides which are directly conjugated to the protein carrier CRM_{197} to form the glycoconjugate. This is effected by reductive amination. CRM_{197} is a nontoxic variant of diphtheria toxin isolated from cultures of *Corynebacterium diphtheriae* strain C7 (ß197) grown in a casamino acids and yeast extract-based medium. CRM_{197} is purified through ultrafiltration, ammonium sulfate precipitation, and ion-exchange chromatography. The individual glycoconjugates are purified by ultrafiltration and column chromatography and are analyzed for saccharide to protein ratios, molecular size, free saccharide, and free protein.

The individual glycoconjugates are compounded to formulate the vaccine, Prevnar™. Potency of the formulated vaccine is determined by quantification of each of the saccharide antigens, and by the saccharide to protein ratios in the individual glycoconjugates.

Prevnar™ is manufactured as a liquid preparation. Each 0.5 mL dose is formulated to contain: 2 µg of each saccharide for serotypes 4, 9V, 14, 18C, 19F, and 23F, and 4 µg of serotype 6B per dose (16 µg total saccharide); approximately 20 µg of CRM_{197} carrier protein; and 0.125 mg of aluminum per 0.5 mL dose as aluminum phosphate adjuvant.

After shaking, the vaccine is a homogeneous, white suspension.

CLINICAL PHARMACOLOGY

S. pneumoniae is an important cause of morbidity and mortality in persons of all ages worldwide. The organism causes invasive infections, such as bacteremia and meningitis, as well as pneumonia and upper respiratory tract infections including otitis media and sinusitis. In children older than 1 month, *S. pneumoniae* is the most common cause of invasive disease.[1] Data from community-based studies performed between 1986 and 1995, indicate that the overall annual incidence of invasive pneumococcal disease in the United States is an estimated 10 to 30 cases per 100,000 persons, with the highest risk in children aged less than or equal to 2 years of age (140 to 160 cases per 100,000 persons).[2,3,4,5,6] Children in group child care have an increased risk for invasive pneumococcal disease.[7,8] Immunocompromised individuals with neutropenia, asplenia, sickle cell disease, disorders of complement and humoral immunity, human immunodeficiency virus (HIV) infections or chronic underlying disease are also at increased risk for invasive pneumococcal disease.[8] *S. pneumoniae* is the most common cause of bacterial meningitis in the United States.[1] The annual incidence of pneumococcal meningitis in children between 1 to 23 months of age is approximately 7 cases per 100,000 persons.[1] Pneumococcal meningitis in childhood has been associated with 8% mortality and may result in neurological sequelae (25%) and hearing loss (32%) in survivors.[9]

S. pneumoniae is an important cause of acute otitis media, identified in 20 to 40% of middle ear fluid cultures.[10,11] The seven serotypes account for approximately 60% of acute otitis media due to *S. pneumoniae* (12-24% of all acute otitis media).[12] The exact contribution of *S. pneumoniae* to childhood pneumonia is unknown, as it is often not possible to identify the causative organisms. In studies of children less than 5 years of age with community-acquired pneumonia, where diagnosis

was attempted using serological methods, antigen testing, or culture data, 30% of cases were classified as bacterial pneumonia, and 70% of these (21% of total community-acquired pneumonia) were found to be due to *S. pneumoniae*.[13,14]

In the past decade the proportion of *S. pneumoniae* isolates resistant to antibiotics has been on the rise in the United States and worldwide. In a multi-center US surveillance study, the prevalence of penicillin and cephalosporin-nonsusceptible (intermediate or high level resistance) invasive disease isolates from children was 21% (range < 5% to 38% among centers), and 9.3% (range 0-18%), respectively. Over the 3-year surveillance period (1993-1996), there was a 50% increase in penicillin-nonsusceptible *S. pneumoniae* (PNSP) strains and a three-fold rise in cephalosporin-nonsusceptible strains.[8] Although generally less common than PNSP, pneumococci resistant to macrolides and trimethoprin-sulfazoxole have also been observed.[4] Day care attendance, a history of ear infection, and a recent history of antibiotic exposure, have also been associated with invasive infections with PNSP in children 2 months to 59 months of age.[7,8] There has been no difference in mortality associated with PNSP strains.[8,9] However, the American Academy of Pediatrics (AAP) revised the antibiotic treatment guidelines in 1997 in response to the increased prevalence of antibiotic-resistant pneumococci.[15]

Approximately 90 serotypes of *S. pneumoniae* have been identified based on antigenic differences in their capsular polysaccharides. The distribution of serotypes responsible for disease differ with age and geographic location.[16]

Serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F have been responsible for approximately 80% of invasive pneumococcal disease in children < 6 years of age in the United States.[12] These 7 serotypes also accounted for 74% of PNSP and 100% of pneumococci with high level penicillin resistance isolated from children < 6 years with invasive disease during a 1993-1994 surveillance by the Centers for Disease Control.[17]

Results of Clinical Evaluations

Efficacy

Efficacy was assessed in a randomized, double-blinded clinical trial in a multiethnic population at Northern California Kaiser Permanente (NCKP), beginning in October 1995, in which 37,816 infants were randomized to receive either Prevnar™ or a control vaccine (an investigational meningococcal group C conjugate vaccine [MnCC]) at 2, 4, 6, and 12-15 months of age. Prevnar™ was administered to 18,906 children and the control vaccine to 18,910 children. Routinely recommended vaccines were also administered which changed during the trial to reflect changing AAP and Advisory Committee on Immunization Practices (ACIP) recommendations. A planned interim analysis was performed upon accrual of 17 cases of invasive disease due to vaccine-type *S. pneumoniae* (August 1998). Ancillary endpoints for evaluation of efficacy against pneumococcal disease were also assessed in this trial.

Efficacy against invasive disease: Invasive disease was defined as isolation and identification of *S. pneumoniae* from normally sterile body sites in children presenting with an acute illness consistent with pneumococcal disease. Weekly surveillance of listings of cultures from the NCKP Regional Microbiology database was conducted to assure ascertainment of all cases. The primary endpoint was efficacy against invasive pneumococcal disease due to vaccine serotypes. The per protocol analysis of the primary endpoint included cases which occurred ≥ 14 days after the third dose. The intent-to-treat (ITT) analysis included all cases of invasive pneumococcal disease due to vaccine serotypes in children who received at least one dose of vaccine. Secondary analyses of efficacy against all invasive pneumococcal disease, regardless of serotype, were also performed according to these same per protocol and ITT definitions. Results of these analyses are presented in Table 1.

TABLE 1
Efficacy of Prevnar™ Against Invasive Disease Due to *S. pneumoniae* in Cases Accrued From October 15, 1995 Through August 20, 1998[18,19]

	Prevnar™	Control*		
	Number of Cases	Number of Cases	Efficacy	95% CI
Vaccine serotypes				
Per protocol	**0**	**17**	**100%**	**75.4, 100**
Intent-to-treat	**0**	**22**	**100%**	**81.7, 100**
All pneumococcal serotypes				
Per protocol	2	20	90.0%	58.3, 98.9
Intent-to-treat	3	27†	88.9%	63.8, 97.9

* Investigational meningococcal group C conjugate vaccine (MnCC).
† Includes one case in an immunocompromised subject.

All 22 cases of invasive disease due to vaccine serotype strains in the ITT population were bacteremic. In addition, the following diagnoses were also reported: meningitis (2), pneumonia (2), and cellulitis (1).

Preliminary efficacy data through an extended follow-up period to April 20, 1999, resulted in a similar efficacy estimate (Per protocol: 1 case in Prevnar™ group, 39 cases in control group; ITT: 3 cases in Prevnar™ group, 49 cases in the control group).

Immunogenicity

Routine Schedule

Subjects from a subset of selected study sites in the NCKP efficacy study were approached for participation in the immunogenicity portion of the study on a volunteer basis. Immune responses following three or four doses of Prevnar™ or the control vaccine were evaluated in children who received either concurrent Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed and Haemophilus b Conjugate Vaccine (Diphtheria CRM_{197} Protein Conjugate), (DTP-HbOC), or Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP), and Haemophilus b Conjugate Vaccine (Diphtheria CRM_{197} Protein Conjugate), (HbOC) vaccines at 2, 4, and 6 months of age. The use of Hepatitis B (Hep B), Oral Polio Vaccine (OPV), Inactivated Polio Vaccine (IPV), Measles-Mumps-Rubella (MMR), and Varicella vaccines were permitted according to the AAP and ACIP recommendations.

Table 2 presents the geometric mean concentrations (GMC) of pneumococcal antibodies following the third and fourth doses of Prevnar™ or the control vaccine when administered concurrently with DTP-HbOC vaccine in the efficacy study.

TABLE 2
Geometric Mean Concentrations (µg/mL) of Pneumococcal Antibodies Following the Third and Fourth Doses of Prevnar™ or Control* When Administered Concurrently With DTP-HbOC in the Efficacy Study[19]

Serotype	Post dose 3 GMC† (95% CI for Prevnar™)		Post dose 4 GMC‡ (95% CI for Prevnar™)	
	Prevnar™§	Control*	Prevnar™§	Control*
	N=88	N=92	N=68	N=61
4	1.46 (1.19, 1.78)	0.03	2.38 (1.88, 3.03)	0.04
6B	4.70 (3.59, 6.14)	0.08	14.45 (11.17, 18.69)	0.17
9V	1.99 (1.64, 2.42)	0.05	3.51 (2.75, 4.48)	0.06
14	4.60 (3.70, 5.74)	0.05	6.52 (5.18, 8.21)	0.06
18C	2.16 (1.73, 2.69)	0.04	3.43 (2.70, 4.37)	0.07
19F	1.39 (1.16, 1.68)	0.09	2.07 (1.66, 2.57)	0.18
23F	1.85 (1.46, 2.34)	0.05	3.82 (2.85, 5.11)	0.09

* Control was investigational meningococcal group C conjugate vaccine (MnCC).
† Mean age of Prevnar™ group was 7.8 months and of control group was 7.7 months. N is slightly less for some serotypes in each group.
‡ Mean age of Prevnar™ group was 14.2 months and of control group was 14.4 months. N is slightly less for some serotypes in each group.
§ $p<0.001$ when Prevnar™ compared to control for each serotype using a Wilcoxon's test.

In another randomized study (Manufacturing Bridging Study, 118-16), immune responses were evaluated following three doses of Prevnar™ administered concomitantly with DTaP and HbOC vaccines at 2, 4, and 6 months of age, IPV at 2 and 4 months of age, and Hep B at 2 and 6 months of age. The control group received concomitant vaccines only. Table 3 presents the immune responses to pneumococcal polysaccharides observed in both this study and in the subset of subjects from the efficacy study that received concomitant DTaP and HbOC vaccines.

TABLE 3
Geometric Mean Concentrations (µg/mL) of Pneumococcal Antibodies Following the Third Dose of Prevnar™ or Control* When Administered Concurrently With DTaP and HbOC in the Efficacy Study† and Manufacturing Bridging Study [19,20]

	Efficacy Study		Manufacturing Bridging Study	
Serotype	Post dose 3 GMC‡ (95% CI for Prevnar™)		Post dose 3 GMC§ (95% CI for Prevnar™)	
	Prevnar™‖	Control*	Prevnar™‖	Control*
	N=32	N=32	N=159	N=83
4	1.47 (1.08, 2.02)	0.02	2.03 (1.75, 2.37)	0.02
6B	2.18 (1.20, 3.96)	0.06	2.97 (2.43, 3.65)	0.07
9V	1.52 (1.04, 2.22)	0.04	1.18 (1.01, 1.39)	0.04
14	5.05 (3.32, 7.70)	0.04	4.64 (3.80, 5.66)	0.04
18C	2.24 (1.65, 3.02)	0.04	1.96 (1.66, 2.30)	0.04
19F	1.54 (1.09, 2.17)	0.10	1.91 (1.63, 2.25)	0.08
23F	1.48 (0.97, 2.25)	0.05	1.71 (1.44, 2.05)	0.05

* Control in efficacy study was investigational meningococcal group C conjugate vaccine (MnCC) and in Manufacturing Bridging Study was concomitant vaccines only.
† Sufficient data are not available to reliably assess GMCs following 4 doses of Prevnar™ when administered with DTaP in the NCKP efficacy study.
‡ Mean age of the Prevnar™ group was 7.4 months and of the control group was 7.6 months. N is slightly less for some serotypes in each group.
§ Mean age of the Prevnar™ group and the control group was 7.2 months.
‖ $p<0.001$ when Prevnar™ compared to control for each serotype using a Wilcoxon's test in the efficacy study and two-sample t-test in the Manufacturing Bridging Study.

In all studies in which the immune responses to Prevnar™ were contrasted to control, a significant antibody response was seen to all vaccine serotypes following three or four doses, although geometric mean concentrations of antibody varied among serotypes.[18,19,20,21,22,23,24,25] The minimum

serum antibody concentration necessary for protection against invasive pneumococcal disease has not been determined for any serotype.

Prevnar™ induces functional antibodies to all vaccine serotypes, as measured by opsonophagocytosis following three doses.[25]

Previously Unvaccinated Older Infants and Children

To determine an appropriate schedule for children 7 months of age or older at the time of the first immunization with Prevnar™, 483 children in 4 ancillary studies received Prevnar™ at various schedules. GMCs attained using the various schedules among older infants and children were comparable to immune responses of children, who received concomitant DTaP, in the NCKP efficacy study (118-8) after 3 doses for most serotypes, as shown in Table 4. These data support the schedule for previously unvaccinated older infants and children who are beyond the age of the infant schedule. For usage in older infants and children see DOSAGE AND ADMINISTRATION.

TABLE 4
Geometric Mean Concentrations (µg/mL) of Pneumococcal Antibodies Following Immunization of Children From 7 Months Through 9 Years of Age With Prevnar™ [26]

Age group, Vaccinations	Study	Sample Size(s)	4	6B	9V	14	18C	19F	23F
7-11 mo. 3 doses	118-12	22	**2.34**	**3.66**	**2.11**	**9.33**	**2.31**	**1.60**	**2.50**
	118-16	39	**3.60**	**4.63**	**2.04**	**5.48**	**1.98**	**2.15**	**1.93**
12-17 mo. 2 doses	118-15*	82-84†	**3.91**	**4.67**	**1.94**	**6.92**	**2.25**	**3.78**	**3.29**
	118-18	33	**7.02**	**4.25**	**3.26**	**6.31**	**3.60**	**3.29**	**2.92**
18-23 mo. 2 doses	118-15*	52-54†	**3.36**	**4.92**	**1.80**	**6.69**	**2.65**	**3.17**	**2.71**
	118-18	45	**6.85**	**3.71**	**3.86**	**6.48**	**3.42**	**3.86**	**2.75**
24-35 mo. 1 dose	118-18	53	**5.34**	**2.90**	**3.43**	**1.88**	**3.03**	**4.07**	**1.56**
36-59 mo. 1 dose	118-18	52	**6.27**	**6.40**	**4.62**	**5.95**	**4.08**	**6.37**	**2.95**
5-9 yrs. 1 dose	118-18	101	**6.92**	**20.84**	**7.49**	**19.32**	**6.72**	**12.51**	**11.57**
118-8, DTaP	Post dose 3	31-32†	1.47	2.18	1.52	5.05	2.24	1.54	1.48

Bold = GMC not inferior to 118-8, DTaP post dose 3 (one-sided lower limit of the 95% CI of GMC ratio ≥ 0.50).

* Study in Navajo and Apache populations.

† Numbers vary with serotype.

INDICATIONS AND USAGE

Prevnar™ is indicated for active immunization of infants and toddlers against invasive disease caused by *S. pneumoniae* due to capsular serotypes included in the vaccine (4, 6B, 9V, 14, 18C, 19F, and 23F). The routine schedule is 2, 4, 6, and 12-15 months of age. For additional information on usage, see DOSAGE AND ADMINISTRATION.

This vaccine is not intended to be used for treatment of active infection.

As with any vaccine, Prevnar™ may not protect 100% of individuals receiving the vaccine.

CONTRAINDICATIONS

Hypersensitivity to any component of the vaccine, including diphtheria toxoid, is a contraindication to use of this vaccine.

The decision to administer or delay vaccination because of a current or recent febrile illness depends largely on the severity of the symptoms and their etiology. Although a severe or even a moderate

febrile illness is sufficient reason to postpone vaccinations, minor illnesses, such as a mild upper respiratory infection with or without low-grade fever, are not generally contraindications.[27,28]

WARNINGS

THIS VACCINE WILL NOT PROTECT AGAINST *S. PNEUMONIAE* DISEASE OTHER THAN THAT CAUSED BY THE SEVEN SEROTYPES INCLUDED IN THE VACCINE, NOR WILL IT PROTECT AGAINST OTHER MICROORGANISMS THAT CAUSE INVASIVE INFECTION SUCH AS BACTEREMIA AND MENINGITIS.

This vaccine should not be given to infants or children with thrombocytopenia or any coagulation disorder that would contraindicate intramuscular injection unless the potential benefit clearly outweighs the risk of administration. If the decision is made to administer this vaccine to children with coagulation disorders, it should be given with caution. (See DRUG INTERACTIONS).

Immunization with Prevnar™ does not substitute for routine diphtheria immunization.

Healthcare professionals should prescribe and/or administer this product with caution to patients with a possible history of latex sensitivity since the packaging contains dry natural rubber.

PRECAUTIONS

Prevnar™ is for intramuscular use only. Prevnar™ SHOULD UNDER NO CIRCUMSTANCES BE ADMINISTERED INTRAVENOUSLY. The safety and immunogenicity for other routes of administration (e.g. subcutaneous) have not been evaluated.

General

CARE IS TO BE TAKEN BY THE HEALTHCARE PROFESSIONAL FOR THE SAFE AND EFFECTIVE USE OF THIS PRODUCT.

1. PRIOR TO ADMINISTRATION OF ANY DOSE OF THIS VACCINE, THE PARENT OR GUARDIAN SHOULD BE ASKED ABOUT THE PERSONAL HISTORY, FAMILY HISTORY, AND RECENT HEALTH STATUS OF THE VACCINE RECIPIENT. THE HEALTHCARE PROFESSIONAL SHOULD ASCERTAIN PREVIOUS IMMUNIZATION HISTORY, CURRENT HEALTH STATUS, AND OCCURRENCE OF ANY SYMPTOMS AND/OR SIGNS OF AN ADVERSE EVENT AFTER PREVIOUS IMMUNIZATIONS IN THE CHILD TO BE IMMUNIZED, IN ORDER TO DETERMINE THE EXISTENCE OF ANY CONTRAINDICATION TO IMMUNIZATION WITH THIS VACCINE AND TO ALLOW AN ASSESSMENT OF RISKS AND BENEFITS.
2. BEFORE THE ADMINISTRATION OF ANY BIOLOGICAL, THE HEALTHCARE PROFESSIONAL SHOULD TAKE ALL PRECAUTIONS KNOWN FOR THE PREVENTION OF ALLERGIC OR ANY OTHER ADVERSE REACTIONS. This should include a review of the patient's history regarding possible sensitivity; the ready availability of epinephrine 1:1000 and other appropriate agents used for control of immediate allergic reactions; and a knowledge of the recent literature pertaining to use of the biological concerned, including the nature of side effects and adverse reactions that may follow its use.
3. Children with impaired immune responsiveness, whether due to the use of immunosuppressive therapy (including irradiation, corticosteroids, antimetabolites, alkylating agents, and cytotoxic agents), a genetic defect, HIV infection, or other causes, may have reduced antibody response to active immunization.[27,28,29] (See DRUG INTERACTIONS).
4. The use of pneumococcal conjugate vaccine does not replace the use of 23-valent pneumococcal polysaccharide vaccine in children ≥ 24 months of age with sickle cell disease, asplenia, HIV infection, chronic illness or who are immunocompromised. Data on sequential vaccination with Prevnar™ followed by 23-valent pneumococcal polysaccharide vaccine are limited. In a randomized study, 23 children ≥ 2 years of age with sickle cell disease were administered either 2 doses of Prevnar™ followed by a dose of polysaccharide vaccine or a single dose of polysaccharide vaccine alone. In this small study, safety and immune responses with the combined schedule were similar to polysaccharide vaccine alone.[30]
5. Since this product is a suspension containing an aluminum adjuvant, shake vigorously immediately prior to use to obtain a uniform suspension prior to withdrawing the dose.

6. A separate sterile syringe and needle or a sterile disposable unit should be used for each individual to prevent transmission of hepatitis or other infectious agents from one person to another. Needles should be disposed of properly and should not be recapped.
7. Special care should be taken to prevent injection into or near a blood vessel or nerve.
8. Healthcare professionals should prescribe and/or administer this product with caution to patients with a possible history of latex sensitivity since the packaging contains dry natural rubber.

Information for Parents or Guardians

Prior to administration of this vaccine, the healthcare professional should inform the parent, guardian, or other responsible adult of the potential benefits and risks to the patient (see ADVERSE REACTIONS and WARNINGS sections), and the importance of completing the immunization series unless contraindicated. Parents or guardians should be instructed to report any suspected adverse reactions to their healthcare professional. The healthcare professional should provide vaccine information statements prior to each vaccination.

DRUG INTERACTIONS

Children receiving therapy with immunosuppressive agents (large amounts of corticosteroids, antimetabolites, alkylating agents, cytotoxic agents) may not respond optimally to active immunization.[28,29,31,32] (See PRECAUTIONS, General).

As with other intramuscular injections, Prevnar™ should be given with caution to children on anticoagulant therapy.

Simultaneous Administration with Other Vaccines

During clinical studies, Prevnar™ was administered simultaneously with DTP-HbOC or DTaP and HbOC, OPV or IPV, Hep B vaccines, MMR, and Varicella vaccine. Thus, the safety experience with Prevnar™ reflects the use of this product as part of the routine immunization schedule.[19,20,22,23,25]

The immune response to routine vaccines when administered with Prevnar™ (at separate sites) was assessed in 3 clinical studies in which there was a control group for comparison. Results for the concurrent immunizations in infants are shown in Table 5 and for toddlers in Table 6. Enhancement of antibody response to HbOC in the infant series was observed. Some suppression of *Haemophilus influenzae* type b (Hib) response was seen at the 4th dose, but over 97% of children achieved titers ≥ 1 µg/mL. Although some inconsistent differences in response to pertussis antigens were observed, the clinical relevance is unknown. The response to 2 doses of IPV given concomitantly with Prevnar™, assessed 3 months after the second dose, was equivalent to controls for poliovirus Types 2 and 3, but lower for Type 1. MMR and Varicella immunogenicity data from controlled clinical trials with concurrent administration of Prevnar™ are not available.

TABLE 5
Concurrent Administration of Prevnar™ With Other Vaccines to Infants in Non-Efficacy Studies[20,23]

Antigen*	GMC*		% Responders†		Study	Vaccine Schedule‡	N	
	Prevnar™	Control§	Prevnar™	Control§		(mo.)	Prevnar™	Control§
Hib	6.2	4.4	99.5, 88.3	97.0, 88.1	118-12	2,4,6	214	67
Diphtheria	0.9	0.8	100	97.0				
Tetanus	3.5	4.1‖	100	100				
PT	19.1	17.8	74.0	69.7				
FHA	43.8	46.7	66.4	69.7				
Pertactin	40.1	50.9	65.6	77.3				
Fimbriae 2	3.3	4.2	44.7	62.5‖				
Hib	11.9	7.8‖	100, 96.9	98.8, 92.8	118-16	2,4,6	159	83
Hep B	--	--	99.4	96.2	118-16	0,2,6	156	80
IPV Type 1	--	--	89.0	93.6¶	118-16	2,4	156	80
Type 2	--	--	94.2	93.6				
Type 3	--	--	83.8	80.8				

* Hib vaccine was HibTITER®, DTaP vaccine was Acel-Imune®. Hib (µg/mL); Dip, Tet (IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) (units/mL).

† Responders = Hib (≥0.15 µg/mL, ≥1.0 µg/mL); Dip, Tet (≥0.1 IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) [4-fold rise]; IPV (≥1:10); Hep B (≥10 mIU/mL).

‡ Schedule for concurrently administered vaccines; Prevnar™ administered at 2, 4, 6 mos.; blood for antibody assessment attained 1 month after third dose, except for IPV (3 months post-immunization).

§ Concurrent vaccines only.

‖ $p<0.05$ when Prevnar™ compared to control group using the following tests: ANCOVA for GMCs in 118-12; ANOVA for GMCs in 118-16; and Fisher's Exact test for % Responders in 118-12.

¶ Lower bound of 90% CI of difference >10%.

TABLE 6
Concurrent Administration of Prevnar™ With Other Vaccines to Toddlers in a Non-Efficacy Study[22]

Antigen*	GMC*		% Responders†		Study‡	Vaccine Schedule§	N	
	Prevnar™	Control‖	Prevnar™	Control‖		(mo.)	Prevnar™	Control‖
Hib	22.7	47.9¶	100, 97.9	100, 100	118-7	12-15	47	26
Diphtheria	2.0	3.2¶	- -100	100				
Tetanus	14.4	18.8	100	100				
PT	68.6	121.2¶	68.1	73.1				
FHA	29.0	48.2¶	68.1	84.6				
Pertactin	84.4	83.0	83.0	96.2				
Fimbriae 2	5.2	3.8	63.8	50.0				

* Hib vaccine was HibTITER®, DTaP vaccine was Acel-Imune®. Hib (µg/mL); Dip, Tet (IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) (units/mL).
† Responders = Hib (≥0.15 µg/mL, ≥1.0 µg/mL); Dip, Tet (≥0.1 IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) [4-fold rise].
‡ Children received a primary series of DTP-HbOC (Tetramune®).
§ Blood for antibody assessment obtained 1 month after dose.
‖ Concurrent vaccines only.
¶ $p<0.05$ when Prevnar™ compared to control group using a two-sample t-test.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Prevnar™ has not been evaluated for any carcinogenic or mutagenic potential, or impairment of fertility.

PREGNANCY

Pregnancy Category C

Animal reproductive studies have not been conducted with this product. It is not known whether Prevnar™ can cause fetal harm when administered to a pregnant woman or whether it can affect reproductive capacity. This vaccine is not recommended for use in pregnant women.

Nursing Mothers

It is not known whether vaccine antigens or antibodies are excreted in human milk. This vaccine is not recommended for use in a nursing mother.

PEDIATRIC USE

Prevnar™ has been shown to be usually well-tolerated and immunogenic in infants. The safety and effectiveness of Prevnar™ in children below the age of 6 weeks have not been established. Immune responses elicited by Prevnar™ among infants born prematurely have not been studied. See DOSAGE AND ADMINISTRATION for the recommended pediatric dosage.

GERIATRIC USE

This vaccine is NOT recommended for use in adult populations. It is not to be used as a substitute for the pneumococcal polysaccharide vaccine, in geriatric populations.

ADVERSE REACTIONS

The majority of the safety experience with Prevnar™ comes from the NCKP Efficacy Trial in which 17,066 infants received 55,352 doses of Prevnar™, along with other routine childhood vaccines through April 1998 (see CLINICAL PHARMACOLOGY section). The number of Prevnar™ recipi-

ents in the safety analysis differs from the number included in the efficacy analysis due to the different lengths of follow-up for these study endpoints. Safety was monitored in this study using several modalities. Local reactions and systemic events occurring within 48 hours of each dose of vaccine were ascertained by scripted telephone interview on a randomly selected subset of approximately 3,000 children in each vaccine group. The rate of relatively rare events requiring medical attention was evaluated across all doses in all study participants using automated databases. Specifically, rates of hospitalizations within 3, 14, 30, and 60 days of immunization, and of emergency room visits within 3, 14, and 30 days of immunization were assessed and compared between vaccine groups for each diagnosis. Seizures within 3 and 30 days of immunization were ascertained across multiple settings (hospitalizations, emergency room or clinic visits, telephone interviews). Deaths and SIDS were ascertained through April 1999. Hospitalizations due to diabetes, autoimmune disorders, and blood disorders were ascertained through August 1999.
In Tables 7 and 8, the rate of local reactions at the Prevnar™ injection site is compared at each dose to the DTP or DTaP injection site in the same children.

TABLE 7
Percentage of Subjects Reporting Local Reactions Within 2 Days Following Immunization With Prevnar™ and DTP-HbOC* Vaccines at 2, 4, 6, and 12-15 Months of Age[19]

Reaction	Dose 1		Dose 2		Dose 3		Dose 4	
	Prevnar™ Site	DTP-HbOC Site†	Prevnar™ Site	DTP-HbOC Site†	Prevnar™ Site	DTP-HbOC Site†	Prevnar™ Site	DTP-HbOC Site†
	N=2890	N=2890	N=2725	N=2725	N=2538	N=2538	N=599	N=599
Erythema								
Any	12.4	21.9	14.3	25.1	15.2	26.5	12.7	23.4
> 2.4 cm	1.2	4.6	1.0	2.9	2.0	4.4	1.7	6.4
Induration								
Any	10.9	22.4	12.3	23.0	12.8	23.3	11.4	20.5
> 2.4 cm	2.6	7.2	2.4	5.6	2.9	6.7	2.8	7.2
Tenderness								
Any	28.0	36.4	25.2	30.5	25.6	32.8	36.5	45.1
Interfered with limb movement	7.9	10.7	7.4	8.4	7.8	10.0	18.5	22.2

* If Hep B vaccine was administered simultaneously, it was administered into the same limb as the DTP-HbOC vaccine. If reactions occurred at either or both sites on that limb, the more severe reaction was recorded.
† $p<0.05$ when Prevnar™ site compared to the DTP-HbOC site using the sign test.

TABLE 8
Percentage of Subjects Reporting Local Reactions Within 2 Days Following Immunization With Prevnar™* and DTaP Vaccines† at 2, 4, 6, and 12-15 Months of Age[19]

Reaction	Dose 1		Dose 2		Dose 3		Dose 4	
	Prevnar™ Site	DTaP Site	Prevnar™ Site	DTaP Site	Prevnar™ Site	DTaP Site	Prevnar™ Site	DTaP Site‡
	N=693	N=693	N=526	N=526	N=422	N=422	N=165	N=165
Erythema								
Any	10.0	6.7§	11.6	10.5	13.8	11.4	10.9	3.6§
> 2.4 cm	1.3	0.4§	0.6	0.6	1.4	1.0	3.6	0.6
Induration								
Any	9.8	6.6§	12.0	10.5	10.4	10.4	12.1	5.5§
> 2.4 cm	1.6	0.9	1.3	1.7	2.4	1.9	5.5	1.8
Tenderness								
Any	17.9	16.0	19.4	17.3	14.7	13.1	23.3	18.4
Interfered with limb movement	3.1	1.8§	4.1	3.3	2.9	1.9	9.2	8.0

* HbOC was administered in the same limb as Prevnar™. If reactions occurred at either or both sites on that limb, the more severe reaction was recorded.

† If Hep B vaccine was administered simultaneously, it was administered into the same limb as DTaP. If reactions occurred at either or both sites on that limb, the more severe reaction was recorded.

‡ Subjects may have received DTP or a mixed DTP/DTaP regimen for the primary series. Thus, this is the 4th dose of a pertussis vaccine, but not a 4th dose of DTaP.

§ $p<0.05$ when Prevnar™ site compared to DTaP site using the sign test.

Table 9 presents the rates of local reactions in previously unvaccinated older infants and children.

TABLE 9
Percentage of Subjects Reporting Local Reactions Within 3 Days of Immunization in Infants and Children from 7 Months Through 9 Years of Age[26]

Age at 1st Vaccination	7 - 11 Mos.						12 - 23 Mos.			24 - 35 Mos.	36 - 59 Mos.	5 - 9 Yrs.
Study No.	118-12			118-16			118-9*	118-18		118-18	118-18	118-18
Dose Number	1	2	3†	1	2	3†	1	1	2	1	1	1
Number of Subjects	54	51	24	81	76	50	60	114	117	46	48	49
Reaction												
Erythema												
Any	16.7	11.8	20.8	7.4	7.9	14.0	48.3	10.5	9.4	6.5	29.2	24.2
> 2.4 cm‡	1.9	0.0	0.0	0.0	0.0	0.0	6.7	1.8	1.7	0.0	8.3	7.1
Induration												
Any	16.7	11.8	8.3	7.4	3.9	10.0	48.3	8.8	6.0	10.9	22.9	25.5
> 2.4 cm‡	3.7	0.0	0.0	0.0	0.0	0.0	3.3	0.9	0.9	2.2	6.3	9.3
Tenderness												
Any	13.0	11.8	12.5	8.6	10.5	12.0	46.7	25.7	26.5	41.3	58.3	82.8
Interfered with limb movement§	1.9	2.0	4.2	1.2	1.3	0.0	3.3	6.2	8.5	13.0	20.8	39.4

* For 118-9, 2 of 60 subjects were ≥24 months of age.
† For 118-12, dose 3 was administered at 15 - 18 mos. of age. For 118-16, dose 3 was administered at 12 - 15 mos. of age.
‡ For 118-16 and 118-18, ≥2 cm.
§ Tenderness interfering with limb movement.

Tables 10 and 11 present the rates of systemic events observed in the efficacy study when Prevnar™ was administered concomitantly with DTP or DTaP.

TABLE 10
Percentage of Subjects* Reporting Systemic Events Within 2 Days Following Immunization With Prevnar™ or Control† Vaccine Concurrently With DTP-HbOC Vaccine at 2, 4, 6, and 12-15 Months of Age

Reaction	Dose 1		Dose 2		Dose 3		Dose 4	
	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†
	N=2998	N=2982	N=2788	N=2761	N=2596	N=2591	N=709	N=733
Fever								
≥ 38.0°C	33.4	28.7‡	34.7	27.4‡	40.6	32.4‡	41.9	36.9
> 39.0°C	1.3	1.3	3.0	1.6‡	5.3	3.4‡	4.5	4.5
Irritability	71.3	67.9‡	69.4	63.8‡	68.9	61.6‡	72.8	65.8‡
Drowsiness	49.2	50.6	32.5	33.6	25.9	23.4‡	21.3	22.7
Restless Sleep	18.1	17.9	27.3	24.3‡	33.3	30.1‡	29.9	28.0
Decreased Appetite	24.7	23.6	22.8	20.3‡	27.7	25.6	33.0	27.4‡
Vomiting	17.9	14.9‡	16.2	14.4	15.5	12.7‡	9.6	6.8
Diarrhea	12.0	10.7	10.9	9.9	11.5	10.4	12.1	11.2
Rash or Hives	0.7	0.6	0.8	0.8	1.4	1.1	1.4	0.8

* Approximately 90% of subjects received prophylactic or therapeutic antipyretics within 48 hours of each dose.
† Investigational meningococcal group C conjugate vaccine (MnCC).
‡ $p<0.05$ when Prevnar™ compared to control group using a Chi-Square test.

TABLE 11
Percentage of Subjects* Reporting Systemic Events Within 2 Days Following Immunization With Prevnar™ or Control† Vaccine Concurrently With DTaP Vaccine at 2, 4, 6, and 12-15 Months of Age‡‡

Reaction	Dose 1		Dose 2		Dose 3		Dose 4	
	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†
	N=710	N=711	N=559	N=508	N=461	N=414	N=224	N=230
Fever								
≥ 38.0°C	15.1	9.4§	23.9	10.8§	19.1	11.8§	21.0	17.0
> 39.0°C	0.9	0.3	2.5	0.8§	1.7	0.7	1.3	1.7
Irritability	48.0	48.2	58.7	45.3§	51.2	44.8	44.2	42.6
Drowsiness	40.7	42.0	25.6	22.8	19.5	21.9	17.0	16.5
Restless Sleep	15.3	15.1	20.2	19.3	25.2	19.0§	20.2	19.1
Decreased Appetite	17.0	13.5	17.4	13.4	20.7	13.8§	20.5	23.1
Vomiting	14.6	14.5	16.8	14.4	10.4	11.6	4.9	4.8
Diarrhea	11.9	8.4§	10.2	9.3	8.3	9.4	11.6	9.2
Rash or Hives	1.4	0.3§	1.3	1.4	0.4	0.5	0.5	1.7

* Approximately 75% of subjects received prophylactic or therapeutic antipyretics within 48 hours of each dose.
† Investigational meningococcal group C conjugate vaccine (MnCC).
‡ Most of these children had received DTP for the primary series. Thus, this is a 4th dose of a pertussis vaccine, but not of DTaP.
§ $p<0.05$ when Prevnar™ compared to control group using a Chi-Square test.

Table 12 presents results from a second study (Manufacturing Bridging Study) conducted at Northern California and Denver Kaiser sites, in which children were randomized to receive one of three lots of Prevnar™ with concomitant vaccines including DTaP, or the same concomitant vaccines alone. Information was ascertained by scripted telephone interview, as described above.

TABLE 12
Percentage of Subjects* Reporting Systemic Reactions Within 3 Days Following Immunization With Prevnar™, DTaP, HbOC, Hep B, and IPV vs. Control† In Manufacturing Bridging Study[20]

Reaction	Dose 1		Dose 2		Dose 3	
	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†
	N=498	N=108	N=452	N=99	N=445	N=89
Fever						
≥ 38.0°C	21.9	10.2‡	33.6	17.2‡	28.1	23.6
> 39.0°C	0.8	0.9	3.8	0.0	2.2	0.0
Irritability	59.7	60.2	65.3	52.5‡	54.2	50.6
Drowsiness	50.8	38.9‡	30.3	31.3	21.2	20.2
Decreased Appetite	19.1	15.7	20.6	11.1‡	20.4	9.0‡

* Approximately 72% of subjects received prophylactic or therapeutic antipyrectics within 48 hours of each dose.
† Control group received concomitant vaccines only in the same schedule as the Prevnar™ group (DTaP, HbOC at dose 1, 2, 3; IPV at doses 1 and 2; Hep B at doses 1 and 3).
‡ $p<0.05$ when Prevnar™ compared to control group using Fisher's Exact test.

Fever (≥ 38.0°C) within 48 hours of a vaccine dose was reported by a greater proportion of subjects who received Prevnar™, compared to control (investigational meningococcal group C conjugate vaccine [MnCC]), after each dose when administered concurrently with DTP-HbOC or DTaP in the efficacy study. In the Manufacturing Bridging Study, fever within 48-72 hours was also reported more commonly after each dose compared to infants in the control group who received only recommended vaccines. When administered concurrently with DTaP in either study, fever rates among Prevnar™ recipients ranged from 15% to 34%, and were greatest after the 2nd dose.

Table 13 presents the frequencies of systemic reactions in previously unvaccinated older infants and children.

TABLE 13
Percentage of Subjects Reporting Systemic Reactions Within 3 Days of Immunization in Infants and Children from 7 Months Through 9 Years of Age[25]

Age at 1st Vaccination	7 - 11 Mos.						12 - 23 Mos.			24 - 35 Mos.	36 - 59 Mos.	5 - 9 Yrs.
Study No.	118-12			118-16			118-9*	118-18		118-18	118-18	118-18
Dose Number	1	2	3†	1	2	3†	1	1	2	1	1	1
Number of Subjects	54	51	24	85	80	50	60	120	117	47	52	100
Reaction												
Fever ≥ 38.0°C	20.8	21.6	25.0	17.6	18.8	22.0	36.7	11.7	6.8	14.9	11.5	7.0
> 39.0°C	1.9	5.9	0.0	1.6	3.9	2.6	0.0	4.4	0.0	4.2	2.3	1.2
Fussiness	29.6	39.2	16.7	54.1	41.3	38.0	40.0	37.5	36.8	46.8	34.6	29.3
Drowsiness	11.1	17.6	16.7	24.7	16.3	14.0	13.3	18.3	11.1	12.8	17.3	11.0
Decreased Appetite	9.3	15.7	0.0	15.3	15.0	30.0	25.0	20.8	16.2	23.4	11.5	9.0

* For 118-9, 2 of 60 subjects were ≥24 months of age.
† For 118-12, dose 3 was administered at 15 - 18 mos. of age. For 118-16, dose 3 was administered at 12 - 15 mos. of age.

Of the 17,066 subjects who received at least one dose of Prevnar™ in the efficacy trial, there were 24 hospitalizations (for 29 diagnoses) within 3 days of a dose from October 1995 through April 1998. Diagnoses were as follows: bronchiolitis (5); congenital anomaly (4); elective procedure, UTI (3 each); acute gastroenteritis, asthma, pneumonia (2 each); aspiration, breath holding, influenza, inguinal hernia repair, otitis media, febrile seizure, viral syndrome, well child/reassurance (1 each). There were 162 visits to the emergency room (for 182 diagnoses) within 3 days of a dose from October 1995 through April 1998. Diagnoses were as follows: febrile illness (20); acute gastroenteritis (19); trauma, URI (16 each); otitis media (15); well child (13); irritable child, viral syndrome (10 each); rash (8); croup, pneumonia (6 each); poisoning/ingestion (5); asthma, bronchiolitis (4 each); febrile seizure, UTI (3 each); thrush, wheezing, breath holding, choking, conjunctivitis, inguinal hernia repair, pharyngitis (2 each); colic, colitis, congestive heart failure, elective procedure, hives, influenza, ingrown toenail, local swelling, roseola, sepsis (1 each).[19]

One case of a hypotonic-hyporesponsive episode (HHE) was reported in the efficacy study following Prevnar™ and concurrent DTP vaccines in the study period from October 1995 through April 1998. Two additional cases of HHE were reported in four other studies and these also occurred in children who received Prevnar™ concurrently with DTP vaccine.[22,25]

In the Kaiser efficacy study in which 17,066 children received a total of 55,352 doses of Prevnar™ and 17,080 children received a total of 55,387 doses of the control vaccine (investiga-

tional meningococcal group C conjugate vaccine [MnCC]), seizures were reported in 8 Prevnar™ recipients and 4 control vaccine recipients within 3 days of immunization from October 1995 through April 1998. Of the 8 Prevnar™ recipients, 7 received concomitant DTP-containing vaccines and one received DTaP. Of the 4 control vaccine recipients, 3 received concomitant DTP-containing vaccines and one received DTaP.[19] In the other 4 studies combined, in which 1,102 children were immunized with 3,347 doses of Prevnar™ and 408 children were immunized with 1,310 doses of control vaccine (either investigational meningococcal group C conjugate vaccine [MnCC] or concurrent vaccines), there was one seizure event reported within 3 days of immunization.[23] This subject received Prevnar™ concurrent with DTaP vaccine.

Twelve deaths (5 SIDS and 7 with clear alternative cause) occurred among subjects receiving Prevnar™, of which 11 (4 SIDS and 7 with clear alternative cause) occurred in the Kaiser efficacy study from October 1995 until April 20, 1999. In comparison, 21 deaths (8 SIDS, 12 with clear alternative cause and one SIDS-like death in an older child), occurred in the control vaccine group during the same time period in the efficacy study.[19,20] The number of SIDS deaths in the efficacy study from October 1995 until April 20, 1999 was similar to or lower than the age and season-adjusted expected rate from the California State data from 1995-1997 and are presented in Table 14.

TABLE 14
Age and Season–Adjusted Comparison of SIDS Rates in the NCKP Efficacy Trial With the Expected Rate from the California State Data for 1995-1997[19]

Vaccine	< One Week After Immunization		≤ Two Weeks After Immunization		≤ One Month After Immunization		≤ One Year After Immunization	
	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs
Prevnar™	1.06	1	2.09	2	4.28	2	8.08	4
Control*	1.06	2	2.09	3†	4.28	3†	8.08	8†

* Investigational meningococcal group C conjugate vaccine (MnCC).
† Does not include one additional case of SIDS-like death in a child older than the usual SIDS age (448 days).

In a review of all hospitalizations that occurred between October 1995 and August 1999 in the efficacy study for the specific diagnoses of aplastic anemia, autoimmune disease, autoimmune hemolytic anemia, diabetes mellitus, neutropenia, and thrombocytopenia, the numbers of such cases were either equal to or less than the expected numbers based on the 1995 Kaiser Vaccine Safety Data Link (VSD) data set.

Overall, the safety of Prevnar™ was evaluated in a total of five clinical studies in which 18,168 infants and children received a total of 58,699 doses of vaccine at 2, 4, 6, and 12-15 months of age. In addition, the safety of Prevnar™ was evaluated in 560 children from 4 ancillary studies who started immunization at 7 months to 9 years of age. Tables 15 and 16 summarize systemic reactogenicity data within 2 or 3 days across 4,748 subjects (13,039 infant doses and 1,706 toddler doses) for whom these data were collected and according to the pertussis vaccine administered concurrently.

TABLE 15
Overall Percentage of Doses Associated With Systemic Events Within 2 or 3 Days For Efficacy Study and All Ancillary Studies When Prevnar™ Administered To Infants As a Primary Series at 2, 4, and 6 Months of Age[19,20,22,23,25]

Systemic Event	Prevnar™ Concurrently With DTP-HbOC (9,191 Doses)*	Prevnar™ Concurrently With DTaP and HbOC (3,848 Doses)†	DTaP and HbOC Control (538 Doses)‡
Fever			
≥ 38.0°C	35.6	21.1	14.2
> 39.0°C	3.1	1.8	0.4
Irritability	69.1	52.5	45.2
Drowsiness	36.9	32.9	27.7
Restless Sleep	25.8	20.6	22.3
Decreased Appetite	24.7	18.1	13.6
Vomiting	16.2	13.4	9.8
Diarrhea	11.4	9.8	4.4
Rash or Hives	0.9	0.6	0.3

* Total from which reaction data are available varies between reactions from 8,874-9,191 doses. Data from studies 118-3, 118-7, 118-8.
† Total from which reaction data are available varies between reactions from 3,121-3,848 doses. Data from studies 118-8, 118-12, 118-16.
‡ Total from which reaction data are available varies between reactions from 295-538 doses. Data from studies 118-12 and 118-16.

TABLE 16
Overall Percentage of Doses Associated With Systemic Events Within 2 or 3 Days For Efficacy Study and All Ancillary Studies When Prevnar™ Administered To Toddlers as a Fourth Dose At 12 to 15 Months of Age[19,22]

Systemic Event	Prevnar™ Concurrently With DTP-HbOC (709 Doses)*	Prevnar™ Concurrently With DTaP and HbOC (270 Doses)†	Prevnar™ Only No Concurrent Vaccines (727 Doses)‡
Fever			
≥ 38.0°C	41.9	19.6	13.4
> 39.0°C	4.5	1.5	1.2
Irritability	72.8	45.9	45.8
Drowsiness	21.3	17.5	15.9
Restless Sleep	29.9	21.2	21.2
Decreased Appetite	33.0	21.1	18.3
Vomiting	9.6	5.6	6.3
Diarrhea	12.1	13.7	12.8
Rash or Hives	1.4	0.7	1.2

* Total from which reaction data are available varies between reactions from 706-709 doses. Data from study 118-8.
† Total from which reaction data are available varies between reactions from 269-270 doses. Data from studies 118-7 and 118-8.
‡ Total from which reaction data are available varies between reactions from 725-727 doses. Data from studies 118-7 and 118-8.

With vaccines in general, including Prevnar™, it is not uncommon for patients to note within 48 to 72 hours at or around the injection site the following minor reactions: edema; pain or tenderness; redness, inflammation or skin discoloration; mass; or local hypersensitivity reaction. Such local reactions are usually self-limited and require no therapy.
As with other aluminum-containing vaccines, a nodule may occasionally be palpable at the injection site for several weeks.[33]

ADVERSE EVENT REPORTING

Any suspected adverse events following immunization should be reported by the healthcare professional to the US Department of Health and Human Services (DHHS). The National Vaccine Injury Compensation Program requires that the manufacturer and lot number of the vaccine administered be recorded by the healthcare professional in the vaccine recipient's permanent medical record (or in a permanent office log or file), along with the date of administration of the vaccine and the name, address, and title of the person administering the vaccine.
The US DHHS has established the Vaccine Adverse Event Reporting System (VAERS) to accept all

reports of suspected adverse events after the administration of any vaccine including, but not limited to, the reporting of events required by the National Childhood Vaccine Injury Act of 1986. The FDA web site is: http://www.fda.gov/cber/vaers/vaers.htm.

The VAERS toll-free number for VAERS forms and information is 800-822-7967.[24]

DOSAGE AND ADMINISTRATION

For intramuscular injection only. *Do not inject intravenously.*

The dose is 0.5 mL to be given intramuscularly.

Since this product is a suspension containing an adjuvant, shake vigorously immediately prior to use to obtain a uniform suspension in the vaccine container. The vaccine should not be used if it cannot be resuspended.

After shaking, the vaccine is a homogeneous, white suspension.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration (see DESCRIPTION). This product should not be used if particulate matter or discoloration is found.

The vaccine should be injected intramuscularly. The preferred sites are the anterolateral aspect of the thigh in infants or the deltoid muscle of the upper arm in toddlers and young children. The vaccine should not be injected in the gluteal area or areas where there may be a major nerve trunk and/or blood vessel. Before injection, the skin at the injection site should be cleansed and prepared with a suitable germicide. After insertion of the needle, aspirate and wait to see if any blood appears in the syringe, which will help avoid inadvertent injection into a blood vessel. If blood appears, withdraw the needle and prepare for a new injection at another site.

Vaccine Schedule

For infants, the immunization series of Prevnar™ consists of three doses of 0.5 mL each, at approximately 2-month intervals, followed by a fourth dose of 0.5 mL at 12-15 months of age. The customary age for the first dose is 2 months of age, but it can be given as young as 6 weeks of age. The recommended dosing interval is 4 to 8 weeks. The fourth dose should be administered at least 2 months after the third dose.

Previously Unvaccinated Older Infants and Children

For previously unvaccinated older infants and children, who are beyond the age of the routine infant schedule, the following schedule applies:[25]

Age at First Dose	Total Number of 0.5 mL Doses
7-11 months of age	3*
12-23 months of age	2†
≥ 24 months through 9 years of age	1

* 2 doses at least 4 weeks apart; third dose after the one-year birthday, separated from the second dose by at least 2 months.

† 2 doses at least 2 months apart.

(See CLINICAL PHARMACOLOGY section for the limited available immunogenicity data and ADVERSE EVENTS section for limited safety data corresponding to the previously noted vaccination schedule for older children).

Safety and immunogenicity data are either limited or not available for children in specific high risk groups for invasive pneumococcal disease (e.g., persons with sickle cell disease, asplenia, HIV-infected).

HOW SUPPLIED

Vial, 1 Dose (5 per package) - NDC 0005-1970-67

CPT Code 90669

STORAGE

DO NOT FREEZE. STORE REFRIGERATED, AWAY FROM FREEZER COMPARTMENT, AT 2°C TO 8°C (36°F TO 46°F).

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

TITLE OF THE INVENTION	:	IMMUNOGENIC CONJUGATES OF STREPTOCOCCUS PNEUMONIAL CAPSULAR POLYMER AND TOXIN OR IN TOXOID
PATENT NUMBER	:	5,360,897
FILING DATE	:	January 9, 1992
ISSUE DATE	:	November 1, 1994
INVENTORS	:	Porter W. Anderson and Ronald J. Eby

Commissioner of Patents and Trademarks
Washington D.C. 20231

DECLARATION OF RONALD J. EBY, Ph.D.
IN SUPPORT OF PATENT TERM EXTENSION APPLICATION
FOR THE 5,360,897 PATENT

Sir:

I, Ronald J. Eby, Ph.D., a citizen of the United States, residing at 297 West Squire Drive, Apt. 3, Rochester, New York, 14623, declare and state that:

1. I am a co-inventor of the above-identified patent and am knowledgeable about its contents.

2. I have been actively involved in vaccine research since 1983.

3. I am a Distinguished Research Scientist and Manager of Carbohydrate Chemistry for Wyeth-Lederle Vaccines, a business unit of Wyeth-Ayerst Laboratories, a division of American Home Products Corporation.

4. I was actively involved in the development of the 7-valent *Pneumococcal* polysaccharide-CRM_{197} conjugate vaccine, also known as Prevnar™ vaccine and am familiar with the product.

5. The Prevnar™ vaccine comprises a sterile solution of seven immunogenic conjugates. Polysaccharides of the capsular antigens of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F individually conjugated, via reductive amination, to a bacterial toxoid comprise the seven immunogenic conjugates. The bacterial toxoid of these seven immunogenic conjugates, CRM_{197}, is a nontoxic variant of diphtheria toxin. *See* Prevnar™ vaccine package insert, at page 1, attached as Exhibit 1.

6. The polysaccharides of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 19F and 23F are present in the Prevnar™ vaccine product as intact capsular polymers. These polysaccharides are not treated with acid, base, or other reagent which would generate capsular polymer fragments. The polysaccharide of 18C serotype conjugated to CRM_{197} is not an intact polymer.

7. Prior to the reductive amination, the polysaccharides of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F are oxidized with periodate to introduce at least two aldehydes. The aldehyde groups on the polysaccharides are carbonyl groups. *See* Hawley's Chemical Dictionary, eleventh edition, 1993, page 223, attached as Exhibit 2. Thus, prior to reductive amination, the polysaccharides contain at least two carbonyl groups.

8. The aldehydes/carbonyl groups on the polysaccharides form, via reductive amination, direct covalent linkages with primary amines (ϵ-amino groups of lysine residues) of CRM_{197}. *See* section 1.6.2.1 of the Drug Master File, attached as Exhibit 3.

9. The individual conjugates of the Prevnar™ vaccine product are "cross-linked." The periodate oxidation of the polysaccharide molecules generates multiple aldehydes on each polysaccharide molecule, which react with the multiple amines present on the CRM_{197} molecules. This results in multiple direct covalent linkages. These multiple direct covalent linkages produce cross-linking between the polysaccharides and the CRM_{197} molecules. This cross-linking is evidenced by the fact that the polysaccharide-CRM_{197} conjugate has an increased size/weight over that of the individual polysaccharide and CRM_{197} taken together.

The undersigned further states that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of the United States Code and that such willful false statements may jeopardize the term extension for the above referenced patent.

Date: 4/13/00

Ronald J. Eby, Ph.D.

Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein)

Prevnar™

℞ only

For Intramuscular Injection Only

DESCRIPTION

Prevnar™, Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein), is a sterile solution of saccharides of the capsular antigens of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F individually conjugated to diphtheria CRM_{197} protein. Each serotype is grown in soy peptone broth. The individual polysaccharides are purified through centrifugation, precipitation, ultrafiltration, and column chromatography. The polysaccharides are chemically activated to make saccharides which are directly conjugated to the protein carrier CRM_{197} to form the glycoconjugate. This is effected by reductive amination. CRM_{197} is a nontoxic variant of diphtheria toxin isolated from cultures of *Corynebacterium diphtheriae* strain C7 (ß197) grown in a casamino acids and yeast extract-based medium. CRM_{197} is purified through ultrafiltration, ammonium sulfate precipitation, and ion-exchange chromatography. The individual glycoconjugates are purified by ultrafiltration and column chromatography and are analyzed for saccharide to protein ratios, molecular size, free saccharide, and free protein.

The individual glycoconjugates are compounded to formulate the vaccine, Prevnar™. Potency of the formulated vaccine is determined by quantification of each of the saccharide antigens, and by the saccharide to protein ratios in the individual glycoconjugates.

Prevnar™ is manufactured as a liquid preparation. Each 0.5 mL dose is formulated to contain: 2 µg of each saccharide for serotypes 4, 9V, 14, 18C, 19F, and 23F, and 4 µg of serotype 6B per dose (16 µg total saccharide); approximately 20 µg of CRM_{197} carrier protein; and 0.125 mg of aluminum per 0.5 mL dose as aluminum phosphate adjuvant.

After shaking, the vaccine is a homogeneous, white suspension.

CLINICAL PHARMACOLOGY

S. pneumoniae is an important cause of morbidity and mortality in persons of all ages worldwide. The organism causes invasive infections, such as bacteremia and meningitis, as well as pneumonia and upper respiratory tract infections including otitis media and sinusitis. In children older than 1 month, *S. pneumoniae* is the most common cause of invasive disease.[1] Data from community-based studies performed between 1986 and 1995, indicate that the overall annual incidence of invasive pneumococcal disease in the United States is an estimated 10 to 30 cases per 100,000 persons, with the highest risk in children aged less than or equal to 2 years of age (140 to 160 cases per 100,000 persons).[2,3,4,5,6] Children in group child care have an increased risk for invasive pneumococcal disease.[7,8] Immunocompromised individuals with neutropenia, asplenia, sickle cell disease, disorders of complement and humoral immunity, human immunodeficiency virus (HIV) infections or chronic underlying disease are also at increased risk for invasive pneumococcal disease.[8] *S. pneumoniae* is the most common cause of bacterial meningitis in the United States.[1] The annual incidence of pneumococcal meningitis in children between 1 to 23 months of age is approximately 7 cases per 100,000 persons.[1] Pneumococcal meningitis in childhood has been associated with 8% mortality and may result in neurological sequelae (25%) and hearing loss (32%) in survivors.[9]

S. pneumoniae is an important cause of acute otitis media, identified in 20 to 40% of middle ear fluid cultures.[10,11] The seven serotypes account for approximately 60% of acute otitis media due to *S. pneumoniae* (12-24% of all acute otitis media).[12] The exact contribution of *S. pneumoniae* to childhood pneumonia is unknown, as it is often not possible to identify the causative organisms. In studies of children less than 5 years of age with community-acquired pneumonia, where diagnosis

was attempted using serological methods, antigen testing, or culture data, 30% of cases were classified as bacterial pneumonia, and 70% of these (21% of total community-acquired pneumonia) were found to be due to *S. pneumoniae*.[13,14]

In the past decade the proportion of *S. pneumoniae* isolates resistant to antibiotics has been on the rise in the United States and worldwide. In a multi-center US surveillance study, the prevalence of penicillin and cephalosporin-nonsusceptible (intermediate or high level resistance) invasive disease isolates from children was 21% (range < 5% to 38% among centers), and 9.3% (range 0-18%), respectively. Over the 3-year surveillance period (1993-1996), there was a 50% increase in penicillin-nonsusceptible *S. pneumoniae* (PNSP) strains and a three-fold rise in cephalosporin-nonsusceptible strains.[8] Although generally less common than PNSP, pneumococci resistant to macrolides and trimethoprin-sulfazoxole have also been observed.[4] Day care attendance, a history of ear infection, and a recent history of antibiotic exposure, have also been associated with invasive infections with PNSP in children 2 months to 59 months of age.[7,8] There has been no difference in mortality associated with PNSP strains.[8,9] However, the American Academy of Pediatrics (AAP) revised the antibiotic treatment guidelines in 1997 in response to the increased prevalence of antibiotic-resistant pneumococci.[15]

Approximately 90 serotypes of *S. pneumoniae* have been identified based on antigenic differences in their capsular polysaccharides. The distribution of serotypes responsible for disease differ with age and geographic location.[16]

Serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F have been responsible for approximately 80% of invasive pneumococcal disease in children < 6 years of age in the United States.[12] These 7 serotypes also accounted for 74% of PNSP and 100% of pneumococci with high level penicillin resistance isolated from children < 6 years with invasive disease during a 1993-1994 surveillance by the Centers for Disease Control.[17]

Results of Clinical Evaluations

Efficacy

Efficacy was assessed in a randomized, double-blinded clinical trial in a multiethnic population at Northern California Kaiser Permanente (NCKP), beginning in October 1995, in which 37,816 infants were randomized to receive either Prevnar™ or a control vaccine (an investigational meningococcal group C conjugate vaccine [MnCC]) at 2, 4, 6, and 12-15 months of age. Prevnar™ was administered to 18,906 children and the control vaccine to 18,910 children. Routinely recommended vaccines were also administered which changed during the trial to reflect changing AAP and Advisory Committee on Immunization Practices (ACIP) recommendations. A planned interim analysis was performed upon accrual of 17 cases of invasive disease due to vaccine-type *S. pneumoniae* (August 1998). Ancillary endpoints for evaluation of efficacy against pneumococcal disease were also assessed in this trial.

Efficacy against invasive disease: Invasive disease was defined as isolation and identification of *S. pneumoniae* from normally sterile body sites in children presenting with an acute illness consistent with pneumococcal disease. Weekly surveillance of listings of cultures from the NCKP Regional Microbiology database was conducted to assure ascertainment of all cases. The primary endpoint was efficacy against invasive pneumococcal disease due to vaccine serotypes. The per protocol analysis of the primary endpoint included cases which occurred ≥ 14 days after the third dose. The intent-to-treat (ITT) analysis included all cases of invasive pneumococcal disease due to vaccine serotypes in children who received at least one dose of vaccine. Secondary analyses of efficacy against all invasive pneumococcal disease, regardless of serotype, were also performed according to these same per protocol and ITT definitions. Results of these analyses are presented in Table 1.

TABLE 1
Efficacy of Prevnar™ Against Invasive Disease Due to *S. pneumoniae* in Cases Accrued From October 15, 1995 Through August 20, 1998[18,19]

	Prevnar™	Control*		
	Number of Cases	Number of Cases	Efficacy	95% CI
Vaccine serotypes				
Per protocol	**0**	**17**	**100%**	**75.4, 100**
Intent-to-treat	**0**	**22**	**100%**	**81.7, 100**
All pneumococcal serotypes				
Per protocol	2	20	90.0%	58.3, 98.9
Intent-to-treat	3	27†	88.9%	63.8, 97.9

* Investigational meningococcal group C conjugate vaccine (MnCC).
† Includes one case in an immunocompromised subject.

All 22 cases of invasive disease due to vaccine serotype strains in the ITT population were bacteremic. In addition, the following diagnoses were also reported: meningitis (2), pneumonia (2), and cellulitis (1).

Preliminary efficacy data through an extended follow-up period to April 20, 1999, resulted in a similar efficacy estimate (Per protocol: 1 case in Prevnar™ group, 39 cases in control group; ITT: 3 cases in Prevnar™ group, 49 cases in the control group).

Immunogenicity

Routine Schedule

Subjects from a subset of selected study sites in the NCKP efficacy study were approached for participation in the immunogenicity portion of the study on a volunteer basis. Immune responses following three or four doses of Prevnar™ or the control vaccine were evaluated in children who received either concurrent Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed and Haemophilus b Conjugate Vaccine (Diphtheria CRM_{197} Protein Conjugate), (DTP-HbOC), or Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP), and Haemophilus b Conjugate Vaccine (Diphtheria CRM_{197} Protein Conjugate), (HbOC) vaccines at 2, 4, and 6 months of age. The use of Hepatitis B (Hep B), Oral Polio Vaccine (OPV), Inactivated Polio Vaccine (IPV), Measles-Mumps-Rubella (MMR), and Varicella vaccines were permitted according to the AAP and ACIP recommendations.

Table 2 presents the geometric mean concentrations (GMC) of pneumococcal antibodies following the third and fourth doses of Prevnar™ or the control vaccine when administered concurrently with DTP-HbOC vaccine in the efficacy study.

TABLE 2
Geometric Mean Concentrations (µg/mL) of Pneumococcal Antibodies Following the Third and Fourth Doses of Prevnar™ or Control* When Administered Concurrently With DTP-HbOC in the Efficacy Study[19]

Serotype	Post dose 3 GMC† (95% CI for Prevnar™)		Post dose 4 GMC‡ (95% CI for Prevnar™)	
	Prevnar™§	Control*	Prevnar™§	Control*
	N=88	N=92	N=68	N=61
4	1.46 (1.19, 1.78)	0.03	2.38 (1.88, 3.03)	0.04
6B	4.70 (3.59, 6.14)	0.08	14.45 (11.17, 18.69)	0.17
9V	1.99 (1.64, 2.42)	0.05	3.51 (2.75, 4.48)	0.06
14	4.60 (3.70, 5.74)	0.05	6.52 (5.18, 8.21)	0.06
18C	2.16 (1.73, 2.69)	0.04	3.43 (2.70, 4.37)	0.07
19F	1.39 (1.16, 1.68)	0.09	2.07 (1.66, 2.57)	0.18
23F	1.85 (1.46, 2.34)	0.05	3.82 (2.85, 5.11)	0.09

* Control was investigational meningococcal group C conjugate vaccine (MnCC).
† Mean age of Prevnar™ group was 7.8 months and of control group was 7.7 months. N is slightly less for some serotypes in each group.
‡ Mean age of Prevnar™ group was 14.2 months and of control group was 14.4 months. N is slightly less for some serotypes in each group.
§ $p<0.001$ when Prevnar™ compared to control for each serotype using a Wilcoxon's test.

In another randomized study (Manufacturing Bridging Study, 118-16), immune responses were evaluated following three doses of Prevnar™ administered concomitantly with DTaP and HbOC vaccines at 2, 4, and 6 months of age, IPV at 2 and 4 months of age, and Hep B at 2 and 6 months of age. The control group received concomitant vaccines only. Table 3 presents the immune responses to pneumococcal polysaccharides observed in both this study and in the subset of subjects from the efficacy study that received concomitant DTaP and HbOC vaccines.

TABLE 3
Geometric Mean Concentrations (µg/mL) of Pneumococcal Antibodies Following the Third Dose of Prevnar™ or Control* When Administered Concurrently With DTaP and HbOC in the Efficacy Study† and Manufacturing Bridging Study [19,20]

	Efficacy Study		Manufacturing Bridging Study	
Serotype	Post dose 3 GMC‡ (95% CI for Prevnar™)		Post dose 3 GMC§ (95% CI for Prevnar™)	
	Prevnar™‖	Control*	Prevnar™‖	Control*
	N=32	N=32	N=159	N=83
4	1.47 (1.08, 2.02)	0.02	2.03 (1.75, 2.37)	0.02
6B	2.18 (1.20, 3.96)	0.06	2.97 (2.43, 3.65)	0.07
9V	1.52 (1.04, 2.22)	0.04	1.18 (1.01, 1.39)	0.04
14	5.05 (3.32, 7.70)	0.04	4.64 (3.80, 5.66)	0.04
18C	2.24 (1.65, 3.02)	0.04	1.96 (1.66, 2.30)	0.04
19F	1.54 (1.09, 2.17)	0.10	1.91 (1.63, 2.25)	0.08
23F	1.48 (0.97, 2.25)	0.05	1.71 (1.44, 2.05)	0.05

* Control in efficacy study was investigational meningococcal group C conjugate vaccine (MnCC) and in Manufacturing Bridging Study was concomitant vaccines only.
† Sufficient data are not available to reliably assess GMCs following 4 doses of Prevnar™ when administered with DTaP in the NCKP efficacy study.
‡ Mean age of the Prevnar™ group was 7.4 months and of the control group was 7.6 months. N is slightly less for some serotypes in each group.
§ Mean age of the Prevnar™ group and the control group was 7.2 months.
‖ $p<0.001$ when Prevnar™ compared to control for each serotype using a Wilcoxon's test in the efficacy study and two-sample t-test in the Manufacturing Bridging Study.

In all studies in which the immune responses to Prevnar™ were contrasted to control, a significant antibody response was seen to all vaccine serotypes following three or four doses, although geometric mean concentrations of antibody varied among serotypes.[18,19,20,21,22,23,24,25] The minimum

serum antibody concentration necessary for protection against invasive pneumococcal disease has not been determined for any serotype.

Prevnar™ induces functional antibodies to all vaccine serotypes, as measured by opsonophagocytosis following three doses.[25]

Previously Unvaccinated Older Infants and Children

To determine an appropriate schedule for children 7 months of age or older at the time of the first immunization with Prevnar™, 483 children in 4 ancillary studies received Prevnar™ at various schedules. GMCs attained using the various schedules among older infants and children were comparable to immune responses of children, who received concomitant DTaP, in the NCKP efficacy study (118-8) after 3 doses for most serotypes, as shown in Table 4. These data support the schedule for previously unvaccinated older infants and children who are beyond the age of the infant schedule. For usage in older infants and children see DOSAGE AND ADMINISTRATION.

TABLE 4
Geometric Mean Concentrations (µg/mL) of Pneumococcal Antibodies Following Immunization of Children From 7 Months Through 9 Years of Age With Prevnar™ [26]

Age group, Vaccinations	Study	Sample Size(s)	4	6B	9V	14	18C	19F	23F
7-11 mo. 3 doses	118-12	22	**2.34**	**3.66**	**2.11**	**9.33**	**2.31**	**1.60**	**2.50**
	118-16	39	**3.60**	**4.63**	**2.04**	**5.48**	**1.98**	**2.15**	**1.93**
12-17 mo. 2 doses	118-15*	82-84†	**3.91**	**4.67**	**1.94**	**6.92**	**2.25**	**3.78**	**3.29**
	118-18	33	**7.02**	**4.25**	**3.26**	**6.31**	**3.60**	**3.29**	**2.92**
18-23 mo. 2 doses	118-15*	52-54†	**3.36**	**4.92**	**1.80**	**6.69**	**2.65**	**3.17**	**2.71**
	118-18	45	**6.85**	**3.71**	**3.86**	**6.48**	**3.42**	**3.86**	**2.75**
24-35 mo. 1 dose	118-18	53	**5.34**	**2.90**	**3.43**	1.88	**3.03**	**4.07**	**1.56**
36-59 mo. 1 dose	118-18	52	**6.27**	**6.40**	**4.62**	**5.95**	**4.08**	**6.37**	**2.95**
5-9 yrs. 1 dose	118-18	101	**6.92**	**20.84**	**7.49**	**19.32**	**6.72**	**12.51**	**11.57**
118-8, DTaP	Post dose 3	31-32†	1.47	2.18	1.52	5.05	2.24	1.54	1.48

Bold = GMC not inferior to 118-8, DTaP post dose 3 (one-sided lower limit of the 95% CI of GMC ratio ≥ 0.50).

* Study in Navajo and Apache populations.

† Numbers vary with serotype.

INDICATIONS AND USAGE

Prevnar™ is indicated for active immunization of infants and toddlers against invasive disease caused by *S. pneumoniae* due to capsular serotypes included in the vaccine (4, 6B, 9V, 14, 18C, 19F, and 23F). The routine schedule is 2, 4, 6, and 12-15 months of age. For additional information on usage, see DOSAGE AND ADMINISTRATION.

This vaccine is not intended to be used for treatment of active infection.

As with any vaccine, Prevnar™ may not protect 100% of individuals receiving the vaccine.

CONTRAINDICATIONS

Hypersensitivity to any component of the vaccine, including diphtheria toxoid, is a contraindication to use of this vaccine.

The decision to administer or delay vaccination because of a current or recent febrile illness depends largely on the severity of the symptoms and their etiology. Although a severe or even a moderate

febrile illness is sufficient reason to postpone vaccinations, minor illnesses, such as a mild upper respiratory infection with or without low-grade fever, are not generally contraindications.[27,28]

WARNINGS

THIS VACCINE WILL NOT PROTECT AGAINST *S. PNEUMONIAE* DISEASE OTHER THAN THAT CAUSED BY THE SEVEN SEROTYPES INCLUDED IN THE VACCINE, NOR WILL IT PROTECT AGAINST OTHER MICROORGANISMS THAT CAUSE INVASIVE INFECTION SUCH AS BACTEREMIA AND MENINGITIS.

This vaccine should not be given to infants or children with thrombocytopenia or any coagulation disorder that would contraindicate intramuscular injection unless the potential benefit clearly outweighs the risk of administration. If the decision is made to administer this vaccine to children with coagulation disorders, it should be given with caution. (See DRUG INTERACTIONS).

Immunization with Prevnar™ does not substitute for routine diphtheria immunization.

Healthcare professionals should prescribe and/or administer this product with caution to patients with a possible history of latex sensitivity since the packaging contains dry natural rubber.

PRECAUTIONS

Prevnar™ is for intramuscular use only. Prevnar™ SHOULD UNDER NO CIRCUMSTANCES BE ADMINISTERED INTRAVENOUSLY. The safety and immunogenicity for other routes of administration (e.g. subcutaneous) have not been evaluated.

General

CARE IS TO BE TAKEN BY THE HEALTHCARE PROFESSIONAL FOR THE SAFE AND EFFECTIVE USE OF THIS PRODUCT.

1. PRIOR TO ADMINISTRATION OF ANY DOSE OF THIS VACCINE, THE PARENT OR GUARDIAN SHOULD BE ASKED ABOUT THE PERSONAL HISTORY, FAMILY HISTORY, AND RECENT HEALTH STATUS OF THE VACCINE RECIPIENT. THE HEALTHCARE PROFESSIONAL SHOULD ASCERTAIN PREVIOUS IMMUNIZATION HISTORY, CURRENT HEALTH STATUS, AND OCCURRENCE OF ANY SYMPTOMS AND/OR SIGNS OF AN ADVERSE EVENT AFTER PREVIOUS IMMUNIZATIONS IN THE CHILD TO BE IMMUNIZED, IN ORDER TO DETERMINE THE EXISTENCE OF ANY CONTRAINDICATION TO IMMUNIZATION WITH THIS VACCINE AND TO ALLOW AN ASSESSMENT OF RISKS AND BENEFITS.
2. BEFORE THE ADMINISTRATION OF ANY BIOLOGICAL, THE HEALTHCARE PROFESSIONAL SHOULD TAKE ALL PRECAUTIONS KNOWN FOR THE PREVENTION OF ALLERGIC OR ANY OTHER ADVERSE REACTIONS. This should include a review of the patient's history regarding possible sensitivity; the ready availability of epinephrine 1:1000 and other appropriate agents used for control of immediate allergic reactions; and a knowledge of the recent literature pertaining to use of the biological concerned, including the nature of side effects and adverse reactions that may follow its use.
3. Children with impaired immune responsiveness, whether due to the use of immunosuppressive therapy (including irradiation, corticosteroids, antimetabolites, alkylating agents, and cytotoxic agents), a genetic defect, HIV infection, or other causes, may have reduced antibody response to active immunization.[27,28,29] (See DRUG INTERACTIONS).
4. The use of pneumococcal conjugate vaccine does not replace the use of 23-valent pneumococcal polysaccharide vaccine in children ≥ 24 months of age with sickle cell disease, asplenia, HIV infection, chronic illness or who are immunocompromised. Data on sequential vaccination with Prevnar™ followed by 23-valent pneumococcal polysaccharide vaccine are limited. In a randomized study, 23 children ≥ 2 years of age with sickle cell disease were administered either 2 doses of Prevnar™ followed by a dose of polysaccharide vaccine or a single dose of polysaccharide vaccine alone. In this small study, safety and immune responses with the combined schedule were similar to polysaccharide vaccine alone.[30]
5. Since this product is a suspension containing an aluminum adjuvant, shake vigorously immediately prior to use to obtain a uniform suspension prior to withdrawing the dose.

6. A separate sterile syringe and needle or a sterile disposable unit should be used for each individual to prevent transmission of hepatitis or other infectious agents from one person to another. Needles should be disposed of properly and should not be recapped.
7. Special care should be taken to prevent injection into or near a blood vessel or nerve.
8. Healthcare professionals should prescribe and/or administer this product with caution to patients with a possible history of latex sensitivity since the packaging contains dry natural rubber.

Information for Parents or Guardians

Prior to administration of this vaccine, the healthcare professional should inform the parent, guardian, or other responsible adult of the potential benefits and risks to the patient (see ADVERSE REACTIONS and WARNINGS sections), and the importance of completing the immunization series unless contraindicated. Parents or guardians should be instructed to report any suspected adverse reactions to their healthcare professional. The healthcare professional should provide vaccine information statements prior to each vaccination.

DRUG INTERACTIONS

Children receiving therapy with immunosuppressive agents (large amounts of corticosteroids, antimetabolites, alkylating agents, cytotoxic agents) may not respond optimally to active immunization.[28,29,31,32] (See PRECAUTIONS, General).

As with other intramuscular injections, Prevnar™ should be given with caution to children on anticoagulant therapy.

Simultaneous Administration with Other Vaccines

During clinical studies, Prevnar™ was administered simultaneously with DTP-HbOC or DTaP and HbOC, OPV or IPV, Hep B vaccines, MMR, and Varicella vaccine. Thus, the safety experience with Prevnar™ reflects the use of this product as part of the routine immunization schedule.[19,20,22,23,25]

The immune response to routine vaccines when administered with Prevnar™ (at separate sites) was assessed in 3 clinical studies in which there was a control group for comparison. Results for the concurrent immunizations in infants are shown in Table 5 and for toddlers in Table 6. Enhancement of antibody response to HbOC in the infant series was observed. Some suppression of *Haemophilus influenzae* type b (Hib) response was seen at the 4th dose, but over 97% of children achieved titers ≥ 1 µg/mL. Although some inconsistent differences in response to pertussis antigens were observed, the clinical relevance is unknown. The response to 2 doses of IPV given concomitantly with Prevnar™, assessed 3 months after the second dose, was equivalent to controls for poliovirus Types 2 and 3, but lower for Type 1. MMR and Varicella immunogenicity data from controlled clinical trials with concurrent administration of Prevnar™ are not available.

TABLE 5
Concurrent Administration of Prevnar™ With Other Vaccines to Infants in Non-Efficacy Studies[20,23]

Antigen*	GMC*		% Responders†		Study	Vaccine Schedule‡	N	
	Prevnar™	Control§	Prevnar™	Control§		(mo.)	Prevnar™	Control§
Hib	6.2	4.4	99.5, 88.3	97.0, 88.1	118-12	2,4,6	214	67
Diphtheria	0.9	0.8	100	97.0				
Tetanus	3.5	4.1‖	100	100				
PT	19.1	17.8	74.0	69.7				
FHA	43.8	46.7	66.4	69.7				
Pertactin	40.1	50.9	65.6	77.3				
Fimbriae 2	3.3	4.2	44.7	62.5‖				
Hib	11.9	7.8‖	100, 96.9	98.8, 92.8	118-16	2,4,6	159	83
Hep B	--	--	99.4	96.2	118-16	0,2,6	156	80
IPV Type 1	--	--	89.0	93.6¶	118-16	2,4	156	80
Type 2	--	--	94.2	93.6				
Type 3	--	--	83.8	80.8				

* Hib vaccine was HibTITER®, DTaP vaccine was Acel-Imune®. Hib (µg/mL); Dip, Tet (IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) (units/mL).

† Responders = Hib (≥0.15 µg/mL, ≥1.0 µg/mL); Dip, Tet (≥0.1 IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) [4-fold rise]; IPV (≥1:10); Hep B (≥10 mIU/mL).

‡ Schedule for concurrently administered vaccines; Prevnar™ administered at 2, 4, 6 mos.; blood for antibody assessment attained 1 month after third dose, except for IPV (3 months post-immunization).

§ Concurrent vaccines only.

‖ $p<0.05$ when Prevnar™ compared to control group using the following tests: ANCOVA for GMCs in 118-12; ANOVA for GMCs in 118-16; and Fisher's Exact test for % Responders in 118-12.

¶ Lower bound of 90% CI of difference >10%.

TABLE 6
Concurrent Administration of Prevnar™ With Other Vaccines to Toddlers in a Non-Efficacy Study[22]

Antigen*	GMC*		% Responders†		Study‡	Vaccine Schedule§	N	
	Prevnar™	Control‖	Prevnar™	Control‖		(mo.)	Prevnar™	Control‖
Hib	22.7	47.9¶	100, 97.9	100, 100	118-7	12-15	47	26
Diphtheria	2.0	3.2¶	.100	100				
Tetanus	14.4	18.8	100	100				
PT	68.6	121.2¶	68.1	73.1				
FHA	29.0	48.2¶	68.1	84.6				
Pertactin	84.4	83.0	83.0	96.2				
Fimbriae 2	5.2	3.8	63.8	50.0				

* Hib vaccine was HibTITER®, DTaP vaccine was Acel-Imune®. Hib (µg/mL); Dip, Tet (IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) (units/mL).
† Responders = Hib (≥0.15 µg/mL, ≥1.0 µg/mL); Dip, Tet (≥0.1 IU/mL); Pertussis Antigens (PT, FHA, Ptn, Fim) [4-fold rise].
‡ Children received a primary series of DTP-HbOC (Tetramune®).
§ Blood for antibody assessment obtained 1 month after dose.
‖ Concurrent vaccines only.
¶ $p<0.05$ when Prevnar™ compared to control group using a two-sample t-test.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Prevnar™ has not been evaluated for any carcinogenic or mutagenic potential, or impairment of fertility.

PREGNANCY

Pregnancy Category C

Animal reproductive studies have not been conducted with this product. It is not known whether Prevnar™ can cause fetal harm when administered to a pregnant woman or whether it can affect reproductive capacity. This vaccine is not recommended for use in pregnant women.

Nursing Mothers

It is not known whether vaccine antigens or antibodies are excreted in human milk. This vaccine is not recommended for use in a nursing mother.

PEDIATRIC USE

Prevnar™ has been shown to be usually well-tolerated and immunogenic in infants. The safety and effectiveness of Prevnar™ in children below the age of 6 weeks have not been established. Immune responses elicited by Prevnar™ among infants born prematurely have not been studied. See DOSAGE AND ADMINISTRATION for the recommended pediatric dosage.

GERIATRIC USE

This vaccine is NOT recommended for use in adult populations. It is not to be used as a substitute for the pneumococcal polysaccharide vaccine, in geriatric populations.

ADVERSE REACTIONS

The majority of the safety experience with Prevnar™ comes from the NCKP Efficacy Trial in which 17,066 infants received 55,352 doses of Prevnar™, along with other routine childhood vaccines through April 1998 (see CLINICAL PHARMACOLOGY section). The number of Prevnar™ recipi-

ents in the safety analysis differs from the number included in the efficacy analysis due to the different lengths of follow-up for these study endpoints. Safety was monitored in this study using several modalities. Local reactions and systemic events occurring within 48 hours of each dose of vaccine were ascertained by scripted telephone interview on a randomly selected subset of approximately 3,000 children in each vaccine group. The rate of relatively rare events requiring medical attention was evaluated across all doses in all study participants using automated databases. Specifically, rates of hospitalizations within 3, 14, 30, and 60 days of immunization, and of emergency room visits within 3, 14, and 30 days of immunization were assessed and compared between vaccine groups for each diagnosis. Seizures within 3 and 30 days of immunization were ascertained across multiple settings (hospitalizations, emergency room or clinic visits, telephone interviews). Deaths and SIDS were ascertained through April 1999. Hospitalizations due to diabetes, autoimmune disorders, and blood disorders were ascertained through August 1999.
In Tables 7 and 8, the rate of local reactions at the Prevnar™ injection site is compared at each dose to the DTP or DTaP injection site in the same children.

TABLE 7
Percentage of Subjects Reporting Local Reactions Within 2 Days Following Immunization With Prevnar™ and DTP-HbOC* Vaccines at 2, 4, 6, and 12-15 Months of Age[19]

Reaction	Dose 1		Dose 2		Dose 3		Dose 4	
	Prevnar™ Site	DTP-HbOC Site†	Prevnar™ Site	DTP-HbOC Site†	Prevnar™ Site	DTP-HbOC Site†	Prevnar™ Site	DTP-HbOC Site†
	N=2890	N=2890	N=2725	N=2725	N=2538	N=2538	N=599	N=599
Erythema								
Any	12.4	21.9	14.3	25.1	15.2	26.5	12.7	23.4
> 2.4 cm	1.2	4.6	1.0	2.9	2.0	4.4	1.7	6.4
Induration								
Any	10.9	22.4	12.3	23.0	12.8	23.3	11.4	20.5
> 2.4 cm	2.6	7.2	2.4	5.6	2.9	6.7	2.8	7.2
Tenderness								
Any	28.0	36.4	25.2	30.5	25.6	32.8	36.5	45.1
Interfered with limb movement	7.9	10.7	7.4	8.4	7.8	10.0	18.5	22.2

* If Hep B vaccine was administered simultaneously, it was administered into the same limb as the DTP-HbOC vaccine. If reactions occurred at either or both sites on that limb, the more severe reaction was recorded.
† $p<0.05$ when Prevnar™ site compared to the DTP-HbOC site using the sign test.

TABLE 8
Percentage of Subjects Reporting Local Reactions Within 2 Days Following Immunization With Prevnar™* and DTaP Vaccines† at 2, 4, 6, and 12-15 Months of Age[19]

Reaction	Dose 1		Dose 2		Dose 3		Dose 4	
	Prevnar™ Site	DTaP Site	Prevnar™ Site	DTaP Site	Prevnar™ Site	DTaP Site	Prevnar™ Site	DTaP Site‡
	N=693	N=693	N=526	N=526	N=422	N=422	N=165	N=165
Erythema								
Any	10.0	6.7§	11.6	10.5	13.8	11.4	10.9	3.6§
> 2.4 cm	1.3	0.4§	0.6	0.6	1.4	1.0	3.6	0.6
Induration								
Any	9.8	6.6§	12.0	10.5	10.4	10.4	12.1	5.5§
> 2.4 cm	1.6	0.9	1.3	1.7	2.4	1.9	5.5	1.8
Tenderness								
Any	17.9	16.0	19.4	17.3	14.7	13.1	23.3	18.4
Interfered with limb movement	3.1	1.8§	4.1	3.3	2.9	1.9	9.2	8.0

* HbOC was administered in the same limb as Prevnar™. If reactions occurred at either or both sites on that limb, the more severe reaction was recorded.

† If Hep B vaccine was administered simultaneously, it was administered into the same limb as DTaP. If reactions occurred at either or both sites on that limb, the more severe reaction was recorded.

‡ Subjects may have received DTP or a mixed DTP/DTaP regimen for the primary series. Thus, this is the 4th dose of a pertussis vaccine, but not a 4th dose of DTaP.

§ $p<0.05$ when Prevnar™ site compared to DTaP site using the sign test.

Table 9 presents the rates of local reactions in previously unvaccinated older infants and children.

TABLE 9
Percentage of Subjects Reporting Local Reactions Within 3 Days of Immunization in Infants and Children from 7 Months Through 9 Years of Age[26]

Age at 1st Vaccination	7 - 11 Mos.						12 - 23 Mos.			24 - 35 Mos.	36 - 59 Mos.	5 - 9 Yrs.
Study No.	118-12			118-16			118-9*	118-18		118-18	118-18	118-18
Dose Number	1	2	3†	1	2	3†	1	1	2	1	1	1
Number of Subjects	54	51	24	81	76	50	60	114	117	46	48	49
Reaction												
Erythema												
Any	16.7	11.8	20.8	7.4	7.9	14.0	48.3	10.5	9.4	6.5	29.2	24.2
> 2.4 cm‡	1.9	0.0	0.0	0.0	0.0	0.0	6.7	1.8	1.7	0.0	8.3	7.1
Induration												
Any	16.7	11.8	8.3	7.4	3.9	10.0	48.3	8.8	6.0	10.9	22.9	25.5
> 2.4 cm‡	3.7	0.0	0.0	0.0	0.0	0.0	3.3	0.9	0.9	2.2	6.3	9.3
Tenderness												
Any	13.0	11.8	12.5	8.6	10.5	12.0	46.7	25.7	26.5	41.3	58.3	82.8
Interfered with limb movement§	1.9	2.0	4.2	1.2	1.3	0.0	3.3	6.2	8.5	13.0	20.8	39.4

* For 118-9, 2 of 60 subjects were ≥24 months of age.
† For 118-12, dose 3 was administered at 15 - 18 mos. of age. For 118-16, dose 3 was administered at 12 - 15 mos. of age.
‡ For 118-16 and 118-18, ≥2 cm.
§ Tenderness interfering with limb movement.

Tables 10 and 11 present the rates of systemic events observed in the efficacy study when Prevnar™ was administered concomitantly with DTP or DTaP.

TABLE 10
Percentage of Subjects* Reporting Systemic Events Within 2 Days Following Immunization With Prevnar™ or Control† Vaccine Concurrently With DTP-HbOC Vaccine at 2, 4, 6, and 12-15 Months of Age[18]

Reaction	Dose 1		Dose 2		Dose 3		Dose 4	
	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†
	N=2998	N=2982	N=2788	N=2761	N=2596	N=2591	N=709	N=733
Fever								
≥ 38.0°C	33.4	28.7‡	34.7	27.4‡	40.6	32.4‡	41.9	36.9
> 39.0°C	1.3	1.3	3.0	1.6‡	5.3	3.4‡	4.5	4.5
Irritability	71.3	67.9‡	69.4	63.8‡	68.9	61.6‡	72.8	65.8‡
Drowsiness	49.2	50.6	32.5	33.6	25.9	23.4‡	21.3	22.7
Restless Sleep	18.1	17.9	27.3	24.3‡	33.3	30.1‡	29.9	28.0
Decreased Appetite	24.7	23.6	22.8	20.3‡	27.7	25.6	33.0	27.4‡
Vomiting	17.9	14.9‡	16.2	14.4	15.5	12.7‡	9.6	6.8
Diarrhea	12.0	10.7	10.9	9.9	11.5	10.4	12.1	11.2
Rash or Hives	0.7	0.6	0.8	0.8	1.4	1.1	1.4	0.8

* Approximately 90% of subjects received prophylactic or therapeutic antipyretics within 48 hours of each dose.
† Investigational meningococcal group C conjugate vaccine (MnCC).
‡ $p<0.05$ when Prevnar™ compared to control group using a Chi-Square test.

TABLE 11
Percentage of Subjects* Reporting Systemic Events Within 2 Days Following Immunization With Prevnar™ or Control† Vaccine Concurrently With DTaP Vaccine at 2, 4, 6, and 12-15 Months of Age[18]

Reaction	Dose 1		Dose 2		Dose 3		Dose 4	
	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†
	N=710	N=711	N=559	N=508	N=461	N=414	N=224	N=230
Fever								
≥ 38.0°C	15.1	9.4§	23.9	10.8§	19.1	11.8§	21.0	17.0
> 39.0°C	0.9	0.3	2.5	0.8§	1.7	0.7	1.3	1.7
Irritability	48.0	48.2	58.7	45.3§	51.2	44.8	44.2	42.6
Drowsiness	40.7	42.0	25.6	22.8	19.5	21.9	17.0	16.5
Restless Sleep	15.3	15.1	20.2	19.3	25.2	19.0§	20.2	19.1
Decreased Appetite	17.0	13.5	17.4	13.4	20.7	13.8§	20.5	23.1
Vomiting	14.6	14.5	16.8	14.4	10.4	11.6	4.9	4.8
Diarrhea	11.9	8.4§	10.2	9.3	8.3	9.4	11.6	9.2
Rash or Hives	1.4	0.3§	1.3	1.4	0.4	0.5	0.5	1.7

* Approximately 75% of subjects received prophylactic or therapeutic antipyretics within 48 hours of each dose.
† Investigational meningococcal group C conjugate vaccine (MnCC).
‡ Most of these children had received DTP for the primary series. Thus, this is a 4th dose of a pertussis vaccine, but not of DTaP.
§ $p<0.05$ when Prevnar™ compared to control group using a Chi-Square test.

Table 12 presents results from a second study (Manufacturing Bridging Study) conducted at Northern California and Denver Kaiser sites, in which children were randomized to receive one of three lots of Prevnar™ with concomitant vaccines including DTaP, or the same concomitant vaccines alone. Information was ascertained by scripted telephone interview, as described above.

TABLE 12
Percentage of Subjects* Reporting Systemic Reactions Within 3 Days Following Immunization With Prevnar™, DTaP, HbOC, Hep B, and IPV vs. Control† In Manufacturing Bridging Study[20]

Reaction	Dose 1		Dose 2		Dose 3	
	Prevnar™	Control†	Prevnar™	Control†	Prevnar™	Control†
	N=498	N=108	N=452	N=99	N=445	N=89
Fever						
≥ 38.0°C	21.9	10.2‡	33.6	17.2‡	28.1	23.6
> 39.0°C	0.8	0.9	3.8	0.0	2.2	0.0
Irritability	59.7	60.2	65.3	52.5‡	54.2	50.6
Drowsiness	50.8	38.9‡	30.3	31.3	21.2	20.2
Decreased Appetite	19.1	15.7	20.6	11.1‡	20.4	9.0‡

* Approximately 72% of subjects received prophylactic or therapeutic antipyrectics within 48 hours of each dose.
† Control group received concomitant vaccines only in the same schedule as the Prevnar™ group (DTaP, HbOC at dose 1, 2, 3; IPV at doses 1 and 2; Hep B at doses 1 and 3).
‡ $p<0.05$ when Prevnar™ compared to control group using Fisher's Exact test.

Fever (≥ 38.0°C) within 48 hours of a vaccine dose was reported by a greater proportion of subjects who received Prevnar™, compared to control (investigational meningococcal group C conjugate vaccine [MnCC]), after each dose when administered concurrently with DTP-HbOC or DTaP in the efficacy study. In the Manufacturing Bridging Study, fever within 48-72 hours was also reported more commonly after each dose compared to infants in the control group who received only recommended vaccines. When administered concurrently with DTaP in either study, fever rates among Prevnar™ recipients ranged from 15% to 34%, and were greatest after the 2nd dose.

Table 13 presents the frequencies of systemic reactions in previously unvaccinated older infants and children.

TABLE 13
Percentage of Subjects Reporting Systemic Reactions Within 3 Days of Immunization in Infants and Children from 7 Months Through 9 Years of Age[26]

Age at 1st Vaccination	7 - 11 Mos.						12 - 23 Mos.			24 - 35 Mos.	36 - 59 Mos.	5 - 9 Yrs.
Study No.	118-12			118-16			118-9*	118-18		118-18	118-18	118-18
Dose Number	1	2	3†	1	2	3†	1	1	2	1	1	1
Number of Subjects	54	51	24	85	80	50	60	120	117	47	52	100
Reaction												
Fever ≥ 38.0°C	20.8	21.6	25.0	17.6	18.8	22.0	36.7	11.7	6.8	14.9	11.5	7.0
> 39.0°C	1.9	5.9	0.0	1.6	3.9	2.6	0.0	4.4	0.0	4.2	2.3	1.2
Fussiness	29.6	39.2	16.7	54.1	41.3	38.0	40.0	37.5	36.8	46.8	34.6	29.3
Drowsiness	11.1	17.6	16.7	24.7	16.3	14.0	13.3	18.3	11.1	12.8	17.3	11.0
Decreased Appetite	9.3	15.7	0.0	15.3	15.0	30.0	25.0	20.8	16.2	23.4	11.5	9.0

* For 118-9, 2 of 60 subjects were ≥24 months of age.
† For 118-12, dose 3 was administered at 15 - 18 mos. of age. For 118-16, dose 3 was administered at 12 - 15 mos. of age.

Of the 17,066 subjects who received at least one dose of Prevnar™ in the efficacy trial, there were 24 hospitalizations (for 29 diagnoses) within 3 days of a dose from October 1995 through April 1998. Diagnoses were as follows: bronchiolitis (5); congenital anomaly (4); elective procedure, UTI (3 each); acute gastroenteritis, asthma, pneumonia (2 each); aspiration, breath holding, influenza, inguinal hernia repair, otitis media, febrile seizure, viral syndrome, well child/reassurance (1 each). There were 162 visits to the emergency room (for 182 diagnoses) within 3 days of a dose from October 1995 through April 1998. Diagnoses were as follows: febrile illness (20); acute gastroenteritis (19); trauma, URI (16 each); otitis media (15); well child (13); irritable child, viral syndrome (10 each); rash (8); croup, pneumonia (6 each); poisoning/ingestion (5); asthma, bronchiolitis (4 each); febrile seizure, UTI (3 each); thrush, wheezing, breath holding, choking, conjunctivitis, inguinal hernia repair, pharyngitis (2 each); colic, colitis, congestive heart failure, elective procedure, hives, influenza, ingrown toenail, local swelling, roseola, sepsis (1 each).[19]

One case of a hypotonic-hyporesponsive episode (HHE) was reported in the efficacy study following Prevnar™ and concurrent DTP vaccines in the study period from October 1995 through April 1998. Two additional cases of HHE were reported in four other studies and these also occurred in children who received Prevnar™ concurrently with DTP vaccine.[22,25]

In the Kaiser efficacy study in which 17,066 children received a total of 55,352 doses of Prevnar™ and 17,080 children received a total of 55,387 doses of the control vaccine (investiga-

tional meningococcal group C conjugate vaccine [MnCC]), seizures were reported in 8 Prevnar™ recipients and 4 control vaccine recipients within 3 days of immunization from October 1995 through April 1998. Of the 8 Prevnar™ recipients, 7 received concomitant DTP-containing vaccines and one received DTaP. Of the 4 control vaccine recipients, 3 received concomitant DTP-containing vaccines and one received DTaP.[19] In the other 4 studies combined, in which 1,102 children were immunized with 3,347 doses of Prevnar™ and 408 children were immunized with 1,310 doses of control vaccine (either investigational meningococcal group C conjugate vaccine [MnCC] or concurrent vaccines), there was one seizure event reported within 3 days of immunization.[23] This subject received Prevnar™ concurrent with DTaP vaccine.

Twelve deaths (5 SIDS and 7 with clear alternative cause) occurred among subjects receiving Prevnar™, of which 11 (4 SIDS and 7 with clear alternative cause) occurred in the Kaiser efficacy study from October 1995 until April 20, 1999. In comparison, 21 deaths (8 SIDS, 12 with clear alternative cause and one SIDS-like death in an older child), occurred in the control vaccine group during the same time period in the efficacy study.[19,20] The number of SIDS deaths in the efficacy study from October 1995 until April 20, 1999 was similar to or lower than the age and season-adjusted expected rate from the California State data from 1995-1997 and are presented in Table 14.

TABLE 14
Age and Season–Adjusted Comparison of SIDS Rates in the NCKP Efficacy Trial With the Expected Rate from the California State Data for 1995-1997[19]

Vaccine	< One Week After Immunization		≤ Two Weeks After Immunization		≤ One Month After Immunization		≤ One Year After Immunization	
	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs
Prevnar™	1.06	1	2.09	2	4.28	2	8.08	4
Control*	1.06	2	2.09	3†	4.28	3†	8.08	8†

* Investigational meningococcal group C conjugate vaccine (MnCC).
† Does not include one additional case of SIDS-like death in a child older than the usual SIDS age (448 days).

In a review of all hospitalizations that occurred between October 1995 and August 1999 in the efficacy study for the specific diagnoses of aplastic anemia, autoimmune disease, autoimmune hemolytic anemia, diabetes mellitus, neutropenia, and thrombocytopenia, the numbers of such cases were either equal to or less than the expected numbers based on the 1995 Kaiser Vaccine Safety Data Link (VSD) data set.

Overall, the safety of Prevnar™ was evaluated in a total of five clinical studies in which 18,168 infants and children received a total of 58,699 doses of vaccine at 2, 4, 6, and 12-15 months of age. In addition, the safety of Prevnar™ was evaluated in 560 children from 4 ancillary studies who started immunization at 7 months to 9 years of age. Tables 15 and 16 summarize systemic reactogenicity data within 2 or 3 days across 4,748 subjects (13,039 infant doses and 1,706 toddler doses) for whom these data were collected and according to the pertussis vaccine administered concurrently.

TABLE 15
Overall Percentage of Doses Associated With Systemic Events Within 2 or 3 Days For Efficacy Study and All Ancillary Studies When Prevnar™ Administered To Infants As a Primary Series at 2, 4, and 6 Months of Age[19,20,22,23,25]

Systemic Event	Prevnar™ Concurrently With DTP-HbOC (9,191 Doses)*	Prevnar™ Concurrently With DTaP and HbOC (3,848 Doses)†	DTaP and HbOC Control (538 Doses)‡
Fever			
≥ 38.0°C	35.6	21.1	**14.2**
> 39.0°C	3.1	1.8	**0.4**
Irritability	69.1	52.5	**45.2**
Drowsiness	36.9	32.9	**27.7**
Restless Sleep	25.8	20.6	**22.3**
Decreased Appetite	24.7	18.1	**13.6**
Vomiting	16.2	13.4	**9.8**
Diarrhea	11.4	9.8	**4.4**
Rash or Hives	0.9	0.6	**0.3**

* Total from which reaction data are available varies between reactions from 8,874-9,191 doses. Data from studies 118-3, 118-7, 118-8.
† Total from which reaction data are available varies between reactions from 3,121-3,848 doses. Data from studies 118-8, 118-12, 118-16.
‡ Total from which reaction data are available varies between reactions from 295-538 doses. Data from studies 118-12 and 118-16.

TABLE 16
Overall Percentage of Doses Associated With Systemic Events Within 2 or 3 Days For Efficacy Study and All Ancillary Studies When Prevnar™ Administered To Toddlers as a Fourth Dose At 12 to 15 Months of Age[19,22]

Systemic Event	Prevnar™ Concurrently With DTP-HbOC (709 Doses)*	Prevnar™ Concurrently With DTaP and HbOC (270 Doses)†	Prevnar™ Only No Concurrent Vaccines (727 Doses)‡
Fever			
≥ 38.0°C	41.9	19.6	**13.4**
> 39.0°C	4.5	1.5	**1.2**
Irritability	72.8	45.9	**45.8**
Drowsiness	21.3	17.5	**15.9**
Restless Sleep	29.9	21.2	**21.2**
Decreased Appetite	33.0	21.1	**18.3**
Vomiting	9.6	5.6	**6.3**
Diarrhea	12.1	13.7	**12.8**
Rash or Hives	1.4	0.7	**1.2**

* Total from which reaction data are available varies between reactions from 706-709 doses. Data from study 118-8.
† Total from which reaction data are available varies between reactions from 269-270 doses. Data from studies 118-7 and 118-8.
‡ Total from which reaction data are available varies between reactions from 725-727 doses. Data from studies 118-7 and 118-8.

With vaccines in general, including Prevnar™, it is not uncommon for patients to note within 48 to 72 hours at or around the injection site the following minor reactions: edema; pain or tenderness; redness, inflammation or skin discoloration; mass; or local hypersensitivity reaction. Such local reactions are usually self-limited and require no therapy.
As with other aluminum-containing vaccines, a nodule may occasionally be palpable at the injection site for several weeks.[33]

ADVERSE EVENT REPORTING

Any suspected adverse events following immunization should be reported by the healthcare professional to the US Department of Health and Human Services (DHHS). The National Vaccine Injury Compensation Program requires that the manufacturer and lot number of the vaccine administered be recorded by the healthcare professional in the vaccine recipient's permanent medical record (or in a permanent office log or file), along with the date of administration of the vaccine and the name, address, and title of the person administering the vaccine.
The US DHHS has established the Vaccine Adverse Event Reporting System (VAERS) to accept all

reports of suspected adverse events after the administration of any vaccine including, but not limited to, the reporting of events required by the National Childhood Vaccine Injury Act of 1986. The FDA web site is: http://www.fda.gov/cber/vaers/vaers.htm.

The VAERS toll-free number for VAERS forms and information is 800-822-7967.[34]

DOSAGE AND ADMINISTRATION

For intramuscular injection only. *Do not inject intravenously.*

The dose is 0.5 mL to be given intramuscularly.

Since this product is a suspension containing an adjuvant, shake vigorously immediately prior to use to obtain a uniform suspension in the vaccine container. The vaccine should not be used if it cannot be resuspended.

After shaking, the vaccine is a homogeneous, white suspension.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration (see DESCRIPTION). This product should not be used if particulate matter or discoloration is found.

The vaccine should be injected intramuscularly. The preferred sites are the anterolateral aspect of the thigh in infants or the deltoid muscle of the upper arm in toddlers and young children. The vaccine should not be injected in the gluteal area or areas where there may be a major nerve trunk and/or blood vessel. Before injection, the skin at the injection site should be cleansed and prepared with a suitable germicide. After insertion of the needle, aspirate and wait to see if any blood appears in the syringe, which will help avoid inadvertent injection into a blood vessel. If blood appears, withdraw the needle and prepare for a new injection at another site.

Vaccine Schedule

For infants, the immunization series of Prevnar™ consists of three doses of 0.5 mL each, at approximately 2-month intervals, followed by a fourth dose of 0.5 mL at 12-15 months of age. The customary age for the first dose is 2 months of age, but it can be given as young as 6 weeks of age. The recommended dosing interval is 4 to 8 weeks. The fourth dose should be administered at least 2 months after the third dose.

Previously Unvaccinated Older Infants and Children

For previously unvaccinated older infants and children, who are beyond the age of the routine infant schedule, the following schedule applies:[26]

Age at First Dose	Total Number of 0.5 mL Doses
7-11 months of age	3*
12-23 months of age	2†
≥ 24 months through 9 years of age	1

* 2 doses at least 4 weeks apart; third dose after the one-year birthday, separated from the second dose by at least 2 months.

† 2 doses at least 2 months apart.

(See CLINICAL PHARMACOLOGY section for the limited available immunogenicity data and ADVERSE EVENTS section for limited safety data corresponding to the previously noted vaccination schedule for older children).

Safety and immunogenicity data are either limited or not available for children in specific high risk groups for invasive pneumococcal disease (e.g., persons with sickle cell disease, asplenia, HIV-infected).

HOW SUPPLIED

Vial, 1 Dose (5 per package) - NDC 0005-1970-67

CPT Code 90669

STORAGE

DO NOT FREEZE. STORE REFRIGERATED, AWAY FROM FREEZER COMPARTMENT, AT 2°C TO 8°C (36°F TO 46°F).

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Revised by

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and
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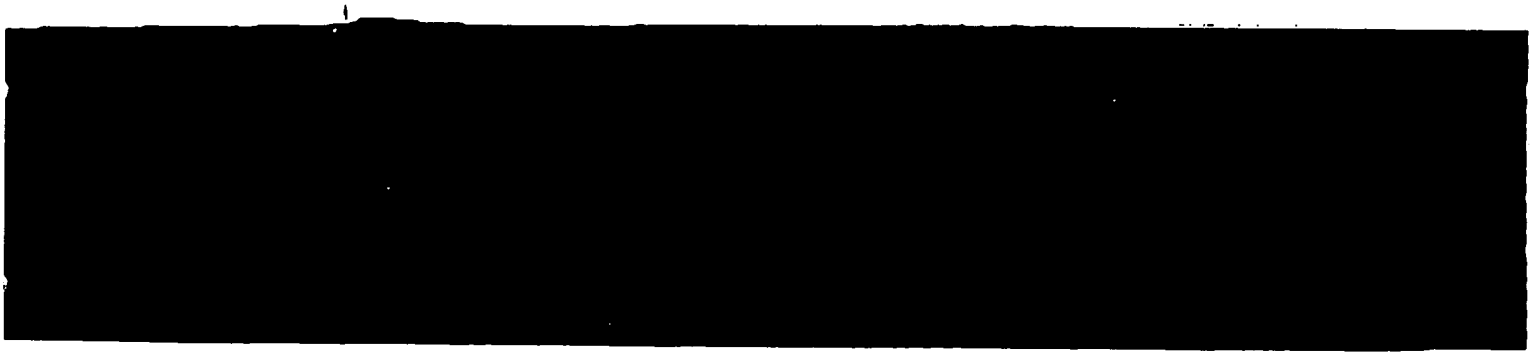

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narcotic in high concentration.
air.
nesis.

e. (tetrachloromethane;
). CAS: 56-23-5.
rinated hydrocarbon.
less liquid; vapor 5.3 times
sweetish, distinctive odor; d
) 76.74C; fp −23.0C; refr index
) press 91.3 mm (20C); wt/gal
sh p none. Miscible with alco-
form, benzene, solvent naphtha
ixed and volatile oils; insoluble
nbustible.
raction of carbon disulfide and
ace of iron; (b) chlorination of
er hydrocarbons at 250–400C.
ation: Treatment with caustic
) remove sulfur chloride, fol-
ation.
CP, electronic.
ingestion, inhalation, and skin
ot use to extinguish fire. nar-
gen (OSHA). TLV: 5 ppm in
to phosgene at high tempera-

Metal degreasing, agricultural
ating organic compounds, pro-
nductors, solvent (fats, oils rub-

ted in products intended for

. See tetrafluoromethane.

See hexachloroethane.

-methoxymetanilic acid)diiso-
ium methoxymetanilate urea).
$_2$Na)NH]$_2$CO.
aste, solids approximately 70%.

See bromophosgene.

See phosgene.

(carbon oxycyanide).

ss liquid. Unstable in the pres-
) −83C, d 1.139 (−114C), mp

LV: (as cyanide) 5 mg/m³ in

nesis.

azole. (N,N-carbonyldiimida-
)bis-1H-imidazole).

$C_7H_6N_4O$. Should be handled in absence of atmospheric moisture to avoid release of carbon dioxide.
Properties: Off-white powder or crystals. Mw 162.15, mp 118–120C.
Use: Enzyme cross-linking agent, condensing agent for nucleoside triphosphate synthesis.

carbonyl fluoride. (fluoroformyl fluoride; carbon oxyfluoride). CAS: 353-50-4. COF_2.
Properties: Colorless, hygroscopic gas. Unstable in the presence of water. Bp −83C, d 1.139 (−114C), fp −114C. Min purity 97 mole %. Nonflammable.
Derivation: Action of silver fluoride on carbon monoxide.
Grade: Technical.
Hazard: Toxic by inhalation, strong irritant to skin. TLV: 2 ppm in air.
Use: Organic synthesis.

carbonyl group. The divalent group $=C=O$ which occurs in a wide range of chemical compounds. It is present in aldehydes, ketones, organic acids, and sugars and in the carboxyl group, i.e.,

In combination with transition metals, it forms coordination compounds which are highly toxic, as they decompose to release carbon monoxide when absorbed by the body, e.g., nickel carbonyl. Several metal carbonyls have antiknock properties. The carbonyl group is also found in combination with nonmetals, as in phosgene (carbonyl chloride); these compounds are also poisonous.

carbonyl sulfide. (carbon oxysulfide).
CAS: 463-58-1. COS.
Properties: Colorless gas with typical sulfide odor except when pure. D gas (air = 1) 2.1, fp −138.8C, bp −50.2C (1 atm). Soluble in water and alcohol.
Derivation: Hydrolysis of ammonium or potassium thiocyanate.
Hazard: Narcotic in high concentrations. Flammable, explosive limits in air 12–28.5%.

carbophenothion. (Generic name for (S-[[p-chlorophenyl]thio]methyl]-O,O-diethyl phosphorodithioate; O-O-diethyl-S-(p-chlorophenylthiomethyl) phosphorodithioate).
$(C_2H_5O)_2P(S)SCH_2S(C_6H_4)Cl$.
Properties: Amber liquid, bp 82C (0.1 mm), d 1.29 (20C). Essentially insoluble in water, miscible in common solvents.
Hazard: Use may be restricted. A cholinesterase inhibitor.
Use: Insecticide, acaricide.

"Carbopol." TM for a group of water-soluble vinyl polymers having excellent suspending, thickening, and gel-forming properties.
Use: Suspensions of glass fibers, graphite, powdered metals; gel formation in hydrocarbons; emulsifier for creosote, tars, and asphalts; lubricants; printing inks; coatings.

carborane. A crystalline compound comprised of boron, carbon, and hydrogen. It can be synthesized in various ways, chiefly by the reaction of a borane (penta- or deca-) with acetylene, either at high-temperature in the gas phase or in the presence of a Lewis base. Alkylated derivatives have been prepared. Carboranes have different structural and chemical characteristics and should not be confused with hydrocarbon derivatives of boron hydrides. The predominant structures are the cage type, the nest type, and the web type, these terms being descriptive of the arrangement of atoms in the crystals. Active research on carborane chemistry has been conducted under sponsorship of the US Office of Naval Research.

"Carbortam." TM for a metallurgical processing alloy containing 15–20% titanium, 6–8% carbon, 2.4–4.0% silicon, 1.75–2.25% boron, and the balance iron except with traces of phosphorus and sulfur. Used to deoxidize and harden steel.

"Carborundum." TM for abrasives and refractories of silicon carbide, fused alumina and other materials.
Properties: For silicon carbide, crystalline form ranges from small to massive crystals in the hexagonal system, the crystals varying from transparent to opaque, with colors from pale green to deep blue or black; hardness 9.17 (Mohs); d 3.06–3.20. Noncombustible, not affected by acids, slowly oxidizes at temperature above 1000C, good heat dissipator, highly refractory. For fused alumina, see properties under the TM "Aloxite."
Use: Abrasive grains and powders for cutting, grinding, and polishing; valve-grinding compounds; grinding wheels; coated abrasive products; antislip tiles and treads; refractory grains.

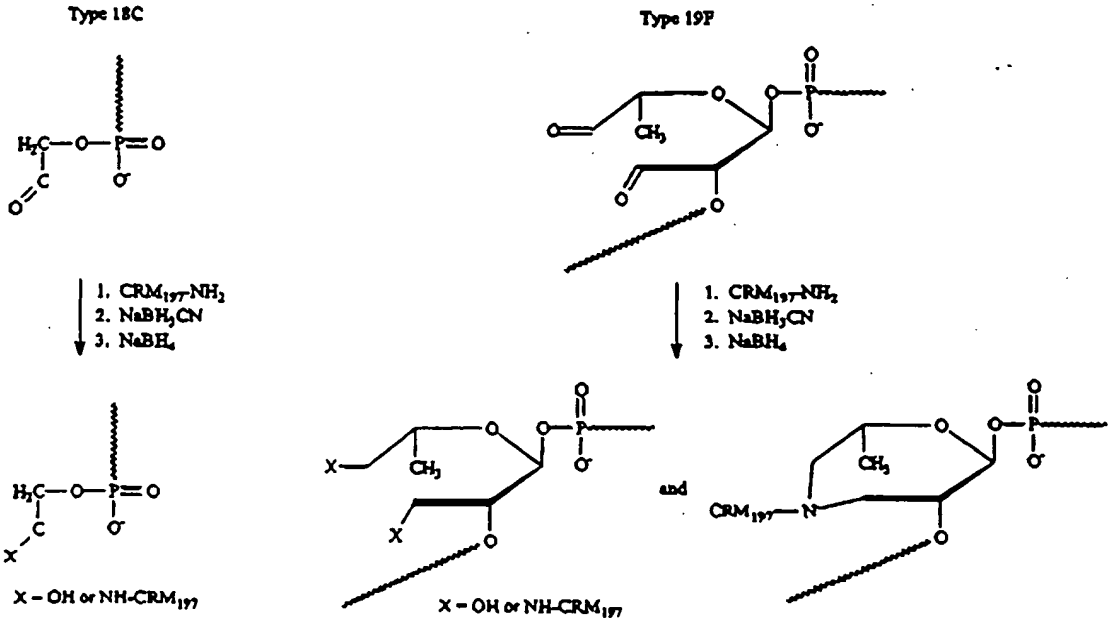
carbosand. Fine sand that has been treated with an organic solution and roasted to produce a material that can be sprayed onto oil slicks to aid in sinking or dispersing them.
See also oil spill treatment.

1.6.2 Pneumococcal Polysaccharide-CRM_{197} Conjugate

1.6.2.1 General Conjugate Structures

Aldehyde groups introduced into the polysaccharide structures by periodate oxidation react with primary amines (ε-amino groups of lysine) from the CRM_{197} to form imines, which are reduced to amines by cyanoborohydride. Single aldehyde groups, such as would form from the glycerol phosphate in serotype 18C or the ribitol in serotype 6B, can react to give secondary amines. Dialdehydes formed from the oxidation of a glycosyl ring have the potential to form tertiary amine ring structures and secondary amines. (King et al. (1975) Arch. Biochem. Biophys. **169**: 464-473 and Sanderson and Wilson (1971) Immunol. **20**: 1061-1065). Examples of these potential conjugate linkage serotypes are depicted in **Figure C-12.**

Figure C-12: Potential Covalent Linkages in Reductively Aminated Pneumoconjugates



1.6.2.2 Serotype 4 Saccharide and Conjugate

a. Serotype 4 Pneumococcal Polysaccharide Chemical Structure

The repeat unit of serotype 4 pneumococcal polysaccharide consists of 2-acetamido-2-deoxy-D-mannopyranosyl, 2-acetamido-2-deoxy-L-fucopyranosyl, 2-acetamido-2-deoxy-D-galactopyranosyl, and D-galactopyranosyl residues, with a pyruvate group attached to the galactose unit as shown in **Figure C-13.**

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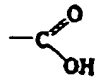
Condensed chemical dictionary.
Hawley's condensed chemical dictionary.

Rev. ed. of: The Condensed chemical dictionary.
10th ed./rev. by Gessner G. Hawley, 1981.
1. Chemistry—Dictionaries. I. Hawley, Gessner Goodrich, 1905– II. Sax, N. Irving (Newton Irving) III. Lewis, Richard J., Sr. IV. Title.
QD5.C5 1987 540'.3'21 86-23333
ISBN 0-442-28097-1

$C_7H_6N_4O$. Should be handled in absence of atmospheric moisture to avoid release of carbon dioxide.
Properties: Off-white powder or crystals. Mw 162.15, mp 118–120C.
Use: Enzyme cross-linking agent, condensing agent for nucleoside triphosphate synthesis.

carbonyl fluoride. (fluoroformyl fluoride; carbon oxyfluoride). CAS: 353-50-4. COF_2.
Properties: Colorless, hygroscopic gas. Unstable in the presence of water. Bp −83C, d 1.139 (−114C), fp −114C. Min purity 97 mole %. Nonflammable.
Derivation: Action of silver fluoride on carbon monoxide.
Grade: Technical.
Hazard: Toxic by inhalation, strong irritant to skin. TLV: 2 ppm in air.
Use: Organic synthesis.

carbonyl group. The divalent group $=C=O$ which occurs in a wide range of chemical compounds. It is present in aldehydes, ketones, organic acids, and sugars and in the carboxyl group, i.e.,



In combination with transition metals, it forms coordination compounds which are highly toxic, as they decompose to release carbon monoxide when absorbed by the body, e.g., nickel carbonyl. Several metal carbonyls have antiknock properties. The carbonyl group is also found in combination with nonmetals, as in phosgene (carbonyl chloride); these compounds are also poisonous.

carbonyl sulfide. (carbon oxysulfide).
CAS: 463-58-1. COS.
Properties: Colorless gas with typical sulfide odor except when pure. D gas (air = 1) 2.1, fp −138.8C, bp −50.2C (1 atm). Soluble in water and alcohol.
Derivation: Hydrolysis of ammonium or potassium thiocyanate.
Hazard: Narcotic in high concentrations. Flammable, explosive limits in air 12–28.5%.

carbophenothion. (Generic name for (S-[[p-chlorophenyl)thio]methyl]-O,O-diethyl phosphorodithioate; O-O-diethyl-S-(p-chlorophenylthiomethyl) phosphorodithioate).
$(C_2H_5O)_2P(S)SCH_2S(C_6H_4)Cl$
Properties: Amber liquid, bp 82C (0.1 mm), d 1.29 (20C). Essentially insoluble in water, miscible in common solvents.
Hazard: Use may be restricted. A cholinesterase inhibitor.
Use: Insecticide, acaricide.

"Carbopol."[7118] TM for a group of water-soluble vinyl polymers having excellent suspending, thickening, and gel-forming properties.
Use: Suspensions of glass fibers, graphite, powdered metals; gel formation in hydrocarbons; emulsifier for creosote, tars, and asphalts; lubricants; printing inks; coatings.

carborane. A crystalline compound comprised of boron, carbon, and hydrogen. It can be synthesized in various ways, chiefly by the reaction of a borane (penta- or deca-) with acetylene, either at high-temperature in the gas phase or in the presence of a Lewis base. Alkylated derivatives have been prepared. Carboranes have different structural and chemical characteristics and should not be confused with hydrocarbon derivatives of boron hydrides. The predominant structures are the cage type, the nest type, and the web type, these terms being descriptive of the arrangement of atoms in the crystals. Active research on carborane chemistry has been conducted under sponsorship of the US Office of Naval Research.

"Carbortam."[7237] TM for a metallurgical processing alloy containing 15–20% titanium, 6–8% carbon, 2.4–4.0% silicon, 1.75–2.25% boron, and the balance iron except with traces of phosphorus and sulfur. Used to deoxidize and harden steel.

"Carborundum."[7280] TM for abrasives and refractories of silicon carbide, fused alumina and other materials.
Properties: For silicon carbide, crystalline form ranges from small to massive crystals in the hexagonal system, the crystals varying from transparent to opaque, with colors from pale green to deep blue or black; hardness 9.17 (Mohs); d 3.06–3.20. Noncombustible, not affected by acids, slowly oxidizes at temperature above 1000C, good heat dissipator, highly refractory. For fused alumina, see properties under the TM "Aloxite."
Use: Abrasive grains and powders for cutting, grinding, and polishing; valve-grinding compounds; grinding wheels; coated abrasive products; antislip tiles and treads; refractory grains.

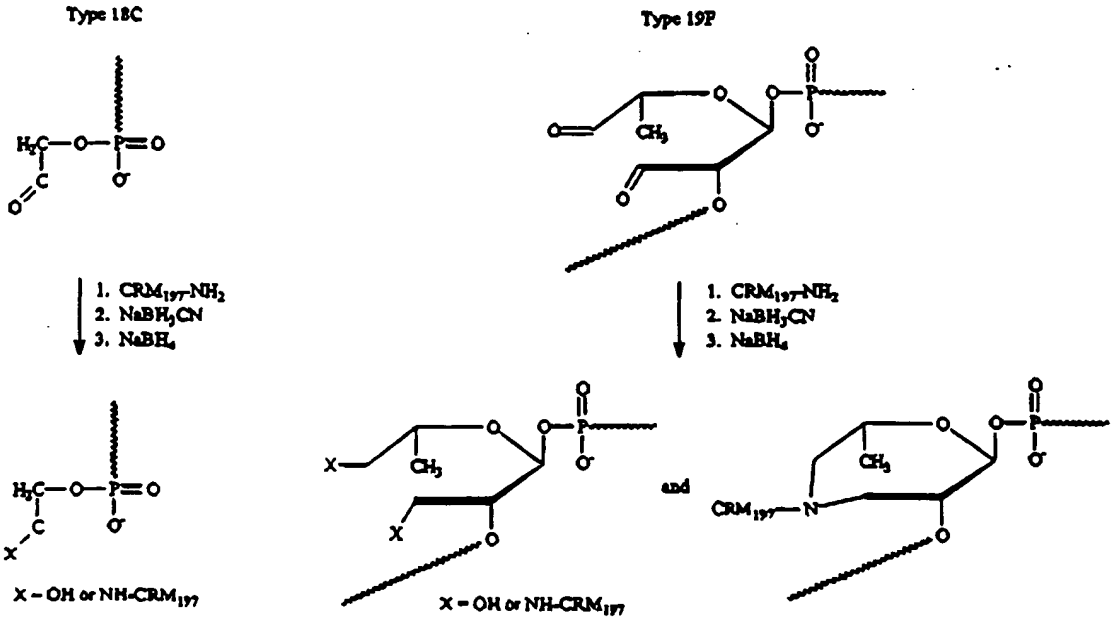
carbosand. Fine sand that has been treated with an organic solution and roasted to produce a material that can be sprayed onto oil slicks to aid in sinking or dispersing them.
See also oil spill treatment.

1.6.2 Pneumococcal Polysaccharide-CRM_{197} Conjugate

1.6.2.1 General Conjugate Structures

Aldehyde groups introduced into the polysaccharide structures by periodate oxidation react with primary amines (ε-amino groups of lysine) from the CRM_{197} to form imines, which are reduced to amines by cyanoborohydride. Single aldehyde groups, such as would form from the glycerol phosphate in serotype 18C or the ribitol in serotype 6B, can react to give secondary amines. Dialdehydes formed from the oxidation of a glycosyl ring have the potential to form tertiary amine ring structures and secondary amines. (King et al. (1975) Arch. Biochem. Biophys. **169:** 464-473 and Sanderson and Wilson (1971) Immunol. **20:** 1061-1065). Examples of these potential conjugate linkage serotypes are depicted in **Figure C-12.**

Figure C-12: Potential Covalent Linkages in Reductively Aminated Pneumoconjugates



1.6.2.2 Serotype 4 Saccharide and Conjugate

a. <u>Serotype 4 Pneumococcal Polysaccharide Chemical Structure</u>

The repeat unit of serotype 4 pneumococcal polysaccharide consists of 2-acetamido-2-deoxy-D-mannopyranosyl, 2-acetamido-2-deoxy-L-fucopyranosyl, 2-acetamido-2-deoxy-D-galactopyranosyl, and D-galactopyranosyl residues, with a pyruvate group attached to the galactose unit as shown in **Figure C-13.**

Lederle—Cont.

Reconstitution Directions for PHARMACY BULK VIAL: Reconstitute the 40 g vial with 172 mL of a suitable diluent (except Lidocaine HCl 0.5% to 1% without epinephrine) listed above to achieve a concentration of 1 g per 5 mL.

Directions for Administration: *Intermittent IV Infusion* — Infuse diluted solution over period of about 30 minutes. During infusion it is desirable to discontinue the primary intravenous solution.

Intravenous Injection (Bolus) — Reconstituted solution should be injected slowly over a 3- to 5-minute period to help avoid vein irritation.

INTRAMUSCULAR ADMINISTRATION (CONVENTIONAL VIALS ONLY):

Reconstitution Directions: Reconstitute each gram of PIPRACIL with 2 mL of a suitable diluent listed above to achieve a concentration of 1 g per 2.5 mL. Shake well until dissolved.

Directions for Administration: When indicated by clinical and bacteriological findings, intramuscular administration of 6 to 8 g daily of PIPRACIL, in divided doses, may be utilized for initiation of therapy. In addition, intramuscular administration of the drug may be considered for maintenance therapy after clinical and bacteriologic improvement has been obtained with intravenous piperacillin sodium treatment. Intramuscular administration should not exceed 2 g per injection at any one site.

The preferred site is the upper outer quadrant of the buttock (ie, gluteus maximus).

The deltoid area should be used only if well-developed, and then only with caution to avoid radial nerve injury. Intramuscular injections should not be made into the lower or mid-third of the upper arm.

STABILITY OF PIPRACIL FOLLOWING RECONSTITUTION:

PIPRACIL is stable in both glass and plastic containers when reconstituted with recommended diluents and when diluted with the intravenous solutions and intravenous admixtures indicated above.

Extensive stability studies have demonstrated chemical stability (potency, pH, and clarity) through 24 hours at room temperature, up to 1 week refrigerated, and up to 1 month frozen (−10° to −20°C). (Note: The 40 g Pharmacy Bulk Vial should not be frozen after reconstitution.) Appropriate consideration of aseptic technique and individual hospital policy, however, may recommend discarding unused portions after storage for 48 hours under refrigeration and discarding after 24 hours storage at room temperature.

ADD-Vantage System:

Stability studies with the ad-mixed ADD-Vantage system have demonstrated chemical stability (potency, pH, and clarity) through 24 hours at room temperature. (Note: The admixed ADD-Vantage should not be refrigerated or frozen after reconstitution.)

Additional stability data available upon request.

HOW SUPPLIED

PIPRACIL *sterile piperacillin sodium* is available in vials containing sterile freeze-dried piperacillin sodium powder equivalent to 2, 3, 4 and 40 g of piperacillin. One gram of piperacillin (as a monosodium salt) contains 1.85 mEq (42.5 mg) of sodium.

Product Numbers:

NDC 0206-3879-16—2 gram/Vial (10s)
NDC 0206-3882-55—3 gram/Vial (10s)
NDC 0206-3880-25—4 gram/Vial (10s)
NDC 0206-3879-47—2 gram infusion Bottle (10s)
NDC 0206-3882-65—3 gram infusion Bottle (10s)
NDC 0206-3880-66—4 gram infusion Bottle (10s)
NDC 0206-3879-27—2 gram ADD-Vantage Vial (10s)
NDC 0206-3882-28—3 gram ADD-Vantage Vial (10s)
NDC 0206-3880-29—4 gram ADD-Vantage Vial (10s)
NDC 0206-3877-60—40 gram Pharmacy Bulk Vial

Military Depot:

NSN 6505-01-137-0039—3 g infusion bottle (10s)

Military and VA Depots:

NSN 6505-01-280-2317—3 g vial (10s)
NSN 6505-01-280-2318—4 g vial (10s)

Store at Controlled Room Temperature 15°–30°C (59°–86°F).

LEDERLE PIPERACILLIN, INC. Rev. 7/88
Carolina, Puerto Rico 00630 24378

Shown in Product Identification Section, page 415

PNU-IMUNE® 23 Ⓑ

[new-i-mune]
Pneumococcal Vaccine, Polyvalent

DESCRIPTION

Pneumococcal Vaccine Polyvalent, PNU-IMUNE 23 is a sterile preparation intended for intramuscular or subcutaneous use. PNU-IMUNE 23 is indicated for immunization against infections caused by the 23 most prevalent types of *Streptococcus pneumoniae* (pneumococci) which are responsible for approximately 90% of serious pneumococcal disease in the United States and worldwide.[1–5] PNU-IMUNE 23 consists of a mixture of purified capsular polysaccharides from types of *S pneumoniae*. [See table below.]

Each of the pneumococcal polysaccharide types is produced separately to assure a high degree of purity. After an individual pneumococcal type is grown, the polysaccharide is separated from the cell and purified by a series of steps including ethanol fractionation. The vaccine is formulated to contain 25 μg of each of the 23 purified polysaccharide types per 0.5 mL dose of vaccine. Thimerosal (a mercury derivative) at a final concentration of 0.01% is added as a preservative.

The vaccine is a clear, colorless liquid.

CLINICAL PHARMACOLOGY

Disease caused by *S pneumoniae* remains an important cause of morbidity and mortality in the United States, particularly in the very young, the elderly, and persons with certain high-risk conditions. Pneumococcal pneumonia accounts for 10% to 25% of all pneumonias and an estimated 40,000 deaths annually.[2]

Studies suggest annual rates of bacteremia of 15 to 19/100,000 for the total population, and 50/100,000 for persons 65 and older. Certain population groups, eg, Native Americans may have considerably higher disease rates.[2]

Mortality from pneumococcal disease is highest in patients with bacteremia or meningitis, patients with underlying medical conditions, and older persons. In some high-risk patients, mortality has been reported to be over 40% for bacteremic disease and 55% for meningitis, despite appropriate antimicrobial therapy.[2]

In addition to the very young and persons 65 years of age or older, patients with certain chronic conditions are at increased risk of developing pneumococcal infection and severe pneumococcal illness. Patients with chronic cardiovascular or pulmonary disease, diabetes mellitus, alcoholism, and cirrhosis are generally immunocompetent but have increased risk. Other patients at greater risk because of decreased responsiveness to polysaccharide antigens or more rapid decline in serum antibody include those with functional or anatomic asplenia (eg, sickle-cell disease or splenectomy), Hodgkin's disease, lymphoma, multiple myeloma, chronic renal failure, nephrotic syndrome, and organ transplantation. Studies indicate that patients with acquired immunodeficiency syndrome (AIDS) are also at increased risk of pneumococcal disease.[6,7] Recurrent pneumococcal meningitis may occur in patients with cerebrospinal fluid leakage that complicates skull fractures or neurologic procedures.

The polysaccharide capsules of pneumococci give these organisms resistance to the phagocytic action of polymorphonuclear leukocytes and monocytes. However, type-specific antibody facilitates their destruction in the body by the mechanism of complement-mediated lysis.

Most healthy adults, including the elderly, demonstrate at least a twofold rise in type-specific antibodies within 2 to 3 weeks of immunization. Similar antibody responses have been reported in patients with alcoholic cirrhosis and diabetes mellitus. In contrast, elderly individuals with chronic pulmonary disease failed to mount a comparable immune response.[26] In immunocompromised patients, the response to immunization may also be lower. Children under 2 years of age respond poorly to most capsular polysaccharide types. Further, response to some pneumococcal types (eg, 6A and 14) important in pediatric infection is decreased in children less than 5 years of age.[8]

In clinical studies with PNU-IMUNE 23 *pneumococcal vaccine, polyvalent* more than 90% of all adults showed twofold or greater increase in geometric mean antibody titer for each capsular type contained in the vaccine.[9]

Patients over the age of 2 years with anatomical or functional asplenia and otherwise intact lymphoid function generally respond to pneumococcal vaccines with a serological conversion comparable to that observed in healthy individuals of the same age.[10]

Patients with acquired immunodeficiency syndrome (AIDS) may have an impaired antibody response to pneumococcal vaccine.[7,11] However, asymptomatic human immunodeficiency virus (HIV)-infected patients, or those with generalized lymphadenopathy, respond to the 23-valent pneumococcal vaccine.[12]

Nomenclature	Pneumococcal Types																					
Danish	1	2	3	4	5	6B	7F	8	9N	9V	10A	11A	12F	14	15B	17F	18C	19F	19A	20	22F	2
U.S.	1	2	3	4	5	26	51	8	9	68	34	43	12	14	54	17	56	19	57	20	22	2

Following immunization of healthy adults, a remain elevated for at least 5 years, but in son these may fall to preimmunization level years.[13,14] A more rapid decline in antibodies children, particularly those who have undergo tomy and those with sickle-cell disease, in wh for some types can fall to preimmunization lev after immunization.[15,16] Similar rates of declin children with nephrotic syndrome.[17]

Controlled clinical trials in South Africa inv gold miners have shown a 6-valent and a 13-va coccal vaccine to be 78.5% effective in pre specific pneumococcal pneumonia and 82.3% preventing pneumococcal bacteremia with t tained in the vaccine.[18] In a preliminary study polysaccharide vaccine in a group consisting with sickle-cell disease and 19 asplenic person no pneumococcal infections in the immun within 2 years of immunization. There were pneumococcal infection in 106 unimmunized, patients with sickle-cell disease. Antibody re asplenic patients was comparable to that controls.[19]

In a study carried out by Austrian and collea valent pneumococcal vaccines prepared for Institute of Allergy and Infectious Disease, the pneumonias caused by the capsular types prese cines was 79%. Reduction in type-specific p bacteremia was 82%.[18]

In a double-blind study of a 14-valent pneumoc carried out in Papua, New Guinea, pneumococ was 84% lower in the immunized group and m pneumonia 44% lower.[20] Five case-control stud have evaluated the efficacy of pneumococcal v prevention of serious pneumococcal disease. studies showed the vaccine to be efficacious, wi mates of efficacy ranging from 61% to 70%.[21–] failed to show efficacy in preventing pneumoc mia.[25] This study was judged inadequate in det vaccination status, and the selection of control ered potentially biased.[2]

A prospective study failed to demonstrate eff pneumococcal pneumonia and bronchitis;[26] t been criticized for methodological flaws.[2] In co spective French study found pneumococcal v 77% effective in reducing the incidence of pneu nursing home residents.[27]

Despite conflicting findings, the data continue t use of pneumococcal vaccine for certain well-de at risk.[2]

INDICATIONS AND USAGE

PNU-IMUNE 23 *pneumococcal vaccine, polyv* cated for immunization against pneumoco caused by those pneumococcal types included in

Adults:

1. All adults 65 or older,[2] with emphasis on imm the older adult while in good health.
2. Immunocompetent adults who are at incre pneumococcal disease or its complications chronic illnesses (eg, cardiovascular or pulmo diabetes mellitus, alcoholism, cirrhosis, or c fluid leaks).[2]
3. Immunocompromised adults at increased risk coccal disease or its complications (eg, splenic or anatomic asplenia, Hodgkin's disease, lym tiple myeloma, chronic renal failure, nephrot or conditions such as organ transplantatio with immunosuppression).[2]

Children:

1. Children 2 years of age or older with chronic cifically associated with increased risk of p disease or its complications (eg, anatomic c asplenia [including sickle-cell disease], ne drome, cerebrospinal fluid leaks, and condi ated with immunosuppression).[2]

Special Groups:

1. Persons living in special environments or so with an identified increased risk of pneumoc or its complications.[2]
2. Patients with acquired immunodeficiency (AIDS) have been shown to have an impair response to pneumococcal vaccine. Howev matic or symptomatic human immunodefi (HIV)-infected patients or those with persist ized lymphadenopathy respond to the 23-val

Timing of Immunization: When elective spl being considered, pneumococcal vaccine should least 2 weeks before surgery, if possible.[2]

For planning cancer chemotherapy or other pressive therapy, the interval between immu

IND CORRESPONDENCE FOR 7-VALENT PNEUMOCOCCAL
CONJUGATE VACCINE
IND NO: 99-0279

Date of Correspondence	Description of Correspondence
08/22/94	Request for meeting
09/07/94	Request for pre-IND meeting
10/26/94	IND submitted
12/01/94	Protocol Amendment: 118-2-1
12/02/94	Information Amendment
12/21/94	Response to FDA Request for information
01/06/95	Protocol Amendment: 118-3
01/13/95	Protocol Amendment: 118-4-1;118-5-1
01/13/95	Cross referencing
01/17/95	Protocol Amendment: 118-3-1
01/20/95	Informational Amendment: Adult Safety Trial Summary
02/03/95	Protocol Amendment: 118-3-4
02/07/95	Protocol Amendment: 118-3-2
02/21/95	Response to PDA request for additional information; clinical manufacturing and preclinical
02/24/95	Protocol Amendment: 118-3-3
03/22/95	Cross reference authorization
04/26/95	Protocol Amendment: 118-3: Correction to Submission
05/02/95	Protocol Amendment: 118-9-1
05/03/95	Pre-Pivotal Meeting
05/05/95	Information Amendment: Interim safety data in infant trial 118-3
05/17/95	Cross referencing in support of clinical trials
05/17/95	Protocol Amendment: 118-6-1
05/18/95	Information Amendment: QA Release Protocol
05/23/95	Protocol Amendment: 118-7-1
05/31/95	Cross-referencing authorization

Date of Correspondence	Description of Correspondence
06/07/95	Cross-referencing
06/07/95	Cross-referencing authorization
06/07/95	Pneumo studies
07/11/95	Pre-Pivotal Trial
08/22/95	Cross Referencing Authorization
08/23/95	Cross Referencing Authorization
08/30/95	Protocol Amendment: 118-8-1
09/26/95	Protocol Amendment: 118-500-1
09/26/95	Cross-Referencing authorization
10/09/95	Efficacy trial
10/11/95	Information Amendment: QA Release Protocol
10/12/95	Response to FDA Request: safety data
10/12/95	Information Amendment: QA Release protocol
10/13/95	Information Amendment: QA Release protocol
10/20/95	Information Amendment: QA Release Protocol
11/21/95	Protocol Amendment: 118-3-1. 118-3-2, 118-3-3
01/08/96	Information Amendment: QA Release Protocol
01/08/96	Protocol Amendment: 118-8
01/23/96	Protocol Amendment: 118-3-2/118-3-3
02/05/96	Information Amendment: QA Release Protocol
03/20/96	Cross-referencing authorization
03/20/96	Protocol Amendment: 118-11
04/05/96	Information Amendment: QA Release Protocol
04/08/96	Requests for Information
04/11/96	Protocol Amendment: 118-7-1
04/23/96	Protocol Amendment; 118-4-1; FDA Forms

Date of Correspondence	Description of Correspondence
04/29/96	Response to FDA Request: Efficacy trial
05/01/96	Protocol Amendment: 118-5-1
05/03/96	Response to FDA Request: 118-3
05/20/96	Notification of Export
06/27/98	118-4-1
07/16/98	118-5-1
08/02/96	Information Amendment: QA Release Protocols
08/09/96	118-4-1
08/13/96	Efficacy Trial
08/29/96	Revised Form 1572
08/29/96	Information Amendment: QA Release Protocol
09/04/96	Protocol 118-11
09/09/96	Protocol Amendment: 118-12-1
09/13/96	Protocol Amendment: 124-2-1
09/13/96	Protocol Amendment: 118-12-4
09/24/98	Protocol Amendment: 118-8
09/26/96	Protocol Amendment: 118-12-2
10/01/96	Information Amendment
10/04/96	Consent Form
10/04/96	Protocol Amendment: 124-2-3
10/15/96	Protocol Amendment: 118-12-3
10/21/96	Export Notification
10/22/96	1995/96 Annual Report
10/30/96	Protocol Amendment: 124-2-2
11/01/96	Compassionate Use
11/26/96	Export Notification

Date of Correspondence	Description of Correspondence
11/26/96	Export Notification
01/03/97	Cross-referencing authorization
01/16/97	Protocol Amendment: Proceedure change
01/28/97	Information Amendment: QA Release Protocols
02/03/97	Protocol Amendment: 118-10-1
02/04/97	Protocol Amendment: 118-12-5
02/25/97	Protocol Amendment: 118-12-4
02/27/97	Compassionate use
03/10/97	Protocol Amendment: 118-15
03/21/97	Protocol: 118-12-3
03/21/97	Protocol: 118-12-2
03/31/97	Protocol: 118-12-5
04/11/97	Response to FDA Request: Efficacy trials
04/17/97	Information Amendment: QA Release Protocol
04/22/97	Meeting
04/23/97	Protocol Amendment: 118-1-1
05/08/97	Protocol Amendment: 118-12-3
05/08/97	Export Request
06/24/97	Revised consent form 118-12-3
06/24/97	118-15
07/03/97	Protocol Amendment: 118-501
07/15/97	Protocol Amendment: 118-8
07/23/97	Export Approval
08/14/97	Protocol Amendment: 118-8
08/14/97	Information Amendment: QA Release Protocol
08/27/97	Request for meeting

Date of Correspondence	Description of Correspondence
10/06/97	Cross-referencing authorization
10/15/97	118-12-3
12/11/97	Protocol Amendment: 118-12
01/26/98	Information Amendment: QA Release Protocol
01/29/98	Overview of meeting
02/02/98	Revised 1572
02/03/98	Protocol Amendment: 118-12-3
02/05/98	Cross-reference
03/17/98	Protocol Amendment: 118-16
03/20/98	Request for Withdrawal of Protocol
03/25/98	Revised Form FDA 1572
04/13/98	Response to FDA Request: 118-16
04/21/98	Pre-FDA meeting
04/22/98	Protocol 124-502
04/22/98	Information Amendment: Revised CIB
04/30/98	Protocol Amendment: 118-15
05/01/98	FDA Questions - Re: Study 118-16
05/15/98	Revised Consent Form & IRB approval
05/21/98	'97 Annual Report
06/08/98	Efficacy trial
06/17/98	Evaluation of Responders
06/18/98	Response to FDA Request: Databases
06/23/98	118-4-1; Revised 1572
07/02/98	Information Amendment: 118-2-1; Clinical Study Summary
07/08/98	Response to FDA Request: Response to CBER questions
07/08/98	Response to FDA Request: Response to CBER questions

Date of Correspondence	Description of Correspondence
07/08/98	Response to FDA Request 118-8-1
07/29/98	118-8-1
07/30/98	118-8-1; Updated analysis for efficacy trials
08/14/98	Response to FDA Request: Response to Correspondence
08/14/98	Response to FDA Request: Response to Correspondence
08/14/98	Study site change
08/17/98	Response to FDA Request: Procedures
08/21/98	Response to FDA Request: Immediate action plan
08/28/98	Subject Study
09/11/98	Media Statement
09/16/98	Subject Study
09/17/98	Information Amendment: Clinical Study Summary for 92-5
09/18/98	Analysis Plan for Study
10/30/98	118-8-1; Final analysis plan
11/06/98	Batch testing results
11/06/98	Analysis Plan
11/18/98	Definitions
11/23/98	Table of Contents for PLA
11/30/98	Response to FDA Request: SOP Information Requested
12/03/98	Pneumonia Analysis Plan for 118-8
12/07/98	Response to FDA Request: Scientific data
12/17/98	Request For Fast Track Designation
12/22/98	Request For Pre-PLA/Pre-ELA meeting
01/06/99	Protocol Amendment: 118-18-1
01/06/99	Protocol Amendment: 118-16-1
01/12/99	Pre-PLA Meeting

Date of Correspondence	Description of Correspondence
01/19/99	Response to FDA Request: Analysis Plan
01/20/99	118-16-1
01/28/99	118-18-1
02/11/99	Bridging study analysis plan
02/19/99	Submissions
03/01/99	Efficacy study
03/08/99	118-8-1
03/08/99	118-8-1
03/25/99	118-16 Analysis Plan
03/27/99	Request for meeting
03/27/99	Assays
04/06/99	1998 Annual Report
04/06/99	Response to FDA Request: Response to questions
04/06/99	Pre-PLA meeting
04/09/99	Response to FDA Request: clinical trial
06/25/99	Protocol Amendment: 118-8
07/12/99	Response to FDA Request: Response to FDA correspondence
07/21/99	Protocol Amendment: 118-18
08/03/99	Cross-Referencing Authorization
08/06/99	118-8
09/23/99	Quality Assurance Lot Release Protocol

PLA CORRESPONDENCE FOR 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE
IND NO: 99-0279

Date of Correspondence	Description of Correspondence
02/26/99	Initiation of rolling PLA
03/04/99	FDA general correspondence: reference number assigned
04/08/99	FDA general correspondence: notification of meeting with FDA
04/12/99	clinical documentation
05/28/99	SAS files
05/31/99	application summary; SAS files; Study report files
06/08/99	SAS files
06/08/99	Request for material
06/18/99	SAS files/immunogenicity data
06/21/99	package insert
06/23/99	package insert
07/01/99	SAS file discussion chronology
07/07/99	safety update submission; efficacy studies
07/13/99	FDA general correspondence: application filed
07/21/99	pending PLA
07/28/99	blood culture isolates
07/28/99	FDA general correspondence: review of submissions
07/30/99	CBER lot release
08/02/99	Attachments
08/06/99	FDA general correspondence: statistical and clinical review of submission
08/09/99	serology
08/13/99	response to FDA questions
08/26/99	FDA general correspondence: labels
08/30/99	response to FDA questions

Date of Correspondence	Description of Correspondence
09/02/99	bulk samples
09/02/99	response to FDA review letter
09/13/99	aluminum concentration determination
09/20/99	labeling
09/21/99	labeling
09/23/99	FDA general correspondence: label and carton
09/25/99	sample SAS program
09/27/99	new product labeling
09/29/99	FDA general correspondence: recommendations
09/29/99	Response to clinical queries
09/30/99	new product labeling
10/04/99	SAS transport files
10/12/99	FDA general correspondence: CBER labeling process
10/13/99	briefing package
10/15/99	confidentiality statement
10/15/99	Request for meeting
10/18/99	clinical queries
10/19/99	safety data
10/21/99	process description
10/25/99	immunogenicity data
10/25/99	blood culture
10/25/99	Time calculations
10/26/99	Indications
10/27/99	Immunogenicity Data

Date of Correspondence	Description of Correspondence
10/28/99	Recommendations
10/29/99	FDA General Correspondence: Meeting Notification
10/29/99	FDA General Correspondence: Meeting agenda
11/02/99	Response to 483 PAI
11/03/99	New Product Labeling
11/10/99	FDA General Correspondence: Notice of meeting cancellation
11/18/99	Trade name
11/29/99	FDA Queries: CBER Response
11/30/99	FDA Queries: CBER comments
11/30/99	FDA Queries: CBER Response
12/03/99	Target dates
12/03/99	Notification of Intent to file PLA Amendment
12/06/99	New Product Labeling
12/07/99	Package insert
12/10/99	Response to 483
12/10/99	Response letter
12/10/99	Product Circular
12/15/99	FDA General Correspondence: labeling
12/16/99	Pre-license inspection
12/17/99	Second set of responses
12/29/98	Pre-license inspection
12/30/99	Statistical and clinical questions
01/07/00	Meeting with FDA
01/11/00	FDA General correspondence: submissions

Date of Correspondence	Description of Correspondence
01/13/00	Response to FDA request
01/21/00	FDA label review
01/27/00	FDA draft summary
01/28/00	Response to general FDA request: summary report
01/29/00	FDA queries: internal committee meeting
01/31/00	Draft summary
01/31/00	Revised product circular
02/01/00	Post-marketing surveillance study
02/01/00	Draft summary
02/01/00	Letter authorizing communication
02/02/00	Updated manufacturing scale stability
02/02/00	Data set
02/02/00	Post-licence
02/03/00	FDA General correspondence: data set
02/09/00	FDA General correspondence: label comments
02/10/00	Expiration dating
02/10/00	FDA General correspondence: label review comments
02/10/00	FDA General correspondence: formatting comments on inserts
02/11/00	Validity Criteria
02/11/00	Package insert
02/12/00	Package insert
02/14/00	Package insert
02/14/00	Post-licensing letters
02/15/00	Stability information

Date of Correspondence	Description of Correspondence
02/15/00	Quality assurance unit
02/15/00	Package insert
02/15/00	FDA General Correspondence: comments
02/15/00	Quality Assurance
02/16/00	Action plan
02/16/00	Package insert
02/17/00	FDA General Correspondence: Approval Letter
03/06/00	Information Amendment
03/28/00	Proposed SAS data set structure

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

POWER OF ATTORNEY
CONCERNING APPLICATION FOR PATENT TERM EXTENSION

I, Egon E. Berg, Vice President and Associate General Counsel, Intellectual Property, of American Home Products Corporation, the undersigned agent for applicant University of Rochester, hereby appoint Estelle J. Tsevdos, Registration No.: 31145 of

KENYON & KENYON
One Broadway
New York, NY 10004

as the attorney to act on its behalf before the U.S. Patent and Trademark Office and to receive all communications and notices relative thereto in connection with the application for patent term extension concerning the below identified patent.

TITLE OF INVENTION	:	IMMUNOGENIC CONJUGATES OF STREPTOCOCCUS PNEUMONIAL CAPSULAR POLYMER AND TOXIN OR IN TOXIAD
PATENT NUMBER	:	5,360,897
FILING DATE	:	January 9, 1992
ISSUE DATE	:	November 1, 1994
INVENTORS	:	Porter W. Anderson and Ronald J. Eby
APPLICANT'S AGENT	:	American Home Products Corporation
ADDRESS	:	American Home Products Corporation Patent Law Department -2B One Campus Drive Parsippany, NJ 07054

DATE: 4-13-00 SIGNATURE: Egon E Berg

Name: Egon E. Berg
Title: Vice President and Associate General Counsel, Intellectual Property

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPOINTMENT OF AGENT
CONCERNING APPLICATION FOR PATENT TERM EXTENSION

I, Mark S. Coburn, Acting Director, Office of Technology Transfer of the University of Rochester, as indicated by the document attached hereto, have authority for the University of Rochester, (See Certificate of Authority, attached hereto), the undersigned applicant for patent term extension, to appoint an agent to further the application for patent term extension concerning the below identified patent. Pursuant to this authority, I hereby appoint American Home Products Corporation, with an office at

One Campus Drive
Parsippany, NJ 07054

as the agent for the University of Rochester to further the application for patent term extension concerning the below identified patent.

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INVENTORS	:	Porter W. Anderson and Ronald J. Eby
APPLICANT	:	University of Rochester
ADDRESS	:	510 Hylan Building RC Box 270140 Rochester, NY 14627-0140

DATE: 4-13-2000 SIGNATURE: Mark S. Coburn

Name: Mark S. Coburn
Title: Acting Director, Office of Technology Transfer of the University of Rochester

UNIVERSITY OF
ROCHESTER

Charles E. Phelps
Provost

April 10, 2000

To Whom it May Concern:

Certificate of Authority

Mark S. Coburn, as Acting Director of the Office of Technology Transfer, is authorized to execute all papers in connection with the procurement and issuance of all patents domestic and foreign on behalf of the University of Rochester

Charles E. Phelps, Provost
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